FOLIAR NEMATODE CONTROL USING NEW NEMATICIDE FORMULATIONS AND ORNAMENTAL PLANT SAFETY ASSOCIATED WITH SEVERAL NEW NEMATICIDES

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Kelsey Mitsuda

Thesis Committee:

Zhiqiang Cheng, Chairperson

Koon-Hui Wang

Brent S. Sipes

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ABSTRACT

Aphelenchoides fragariae is a species of foliar nematode that is an increasingly widespread pathogen of ornamental crops with a wide host range, attacking more than 250 plants species in 47 plant families. The most recognizable field symptom of foliar nematodes is the interveinal lesions on leaves. Previously, chemical treatments using active ingredients such as methyl bromide, oxamyl and parathion were effective against foliar nematodes. However, due to environmental concerns and their high toxicity, these chemicals are no longer available for foliar nematode control. The overall goal of this study is therefore to determine the effectiveness of several new, reduced-risk nematicides against foliar nematodes on certain popular ornamental plants in Hawai‘i. Specific objectives are 1) the efficacy of several newly developed nematicides for managing foliar nematodes on the fern species *Microlepia strigosa*; and 2) if these newly developed nematicides have phytotoxicity effects on ornamental plants commonly used in Hawai‘i's landscape industry; *Microlepia strigosa, Frangipani, Raphiolepsis indica, Hibiscus*, *Phalaenopsis*, and *Anthurium adreanum*. Foliar nematodes were extracted from infected fern tissues using the Baermann funnel technique. These nematodes were cultured in the lab using carrot discs and the cultures were refreshed every 5-7 weeks. New nematicides ESP 715 consisting of fluopyram as the active ingredient (a.i.) along with two other bionematicides, MBI 304 and Majestene, with a.i. of *Chromobacterium* spp. strain extract and *Burkholderia* spp. strain extract, respectively, were tested for potential control of *Aphelenchoides* spp. on *Microlepia strigosa*. Height, width and weight of fern were assessed weekly over 6 weeks after foliar nematode inoculation on the leaves. Foliar nematode damage was assessed at the end of the experiment. In addition, ESP 715, MBI 304 and Majestene were examined for phytotoxicity on *M. strigosa, Frangipani, R. indica, Hibiscus, Phalaenopsis,* and *A. adreanum* at various rates: Fluopyram at 0 ml/L, 0.33 ml/L, 0.66 ml/L and 1.34 ml/L. Except palapalai which was only tested with 2 rates of fluopyram: 0.66ml/L and 1.34 ml/L. Additionally MBI 304 and Majestene were examined for phytotoxiticy on *M. strigosa* plants: MBI 304 at 4,793 mg/L and MBI 205 at 20 ml/L. All plants treated with these nematicides received three applications at 14-day intervals. Untreated plants were included as the control. No visual foliar phytotoxicity symptoms were observed on all treatments throughout the 26-week evaluation period for *Frangipani, R. indica,*

Hibiscus, *Phalaenopsis*, and *A. adraeanum* and the 14-week period for bionematicides on *M. strigosa*., except for fluopyram on *M. strigosa*. Fluopyram at both tested rates caused visual phytotoxicity effects. 0.66 ml/L of fluopyram caused severity ratings of 1.05 on the 0-5 scale. 1.34 ml/L of fluopyram caused severity ratings of 0.95. Severity ratings for both rates of fluopyram were significantly higher than the noninoculated control and significantly lower than the inoculated control. However, fluopyram did not suppress foliar nematodes. *Burkholderia* and *Chromobacterium* did not suppress the number of foliar nematodes significantly but reduced the numbers by 65.7% and 75.8%, respectively. Although various plant growth factors were stunted on hibiscus, orchid, anthurium, indian hawthorn and plumeria by fluopyram, it did not affect the marketability of the plants as no visual foliar phytotoxicity symptom was observed.

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1.1. Foliar nematode *Aphelenchoides fragariae*

Plant-parasitic nematodes are microscopic roundworms that have detrimental effects on their hosts (Handoo, 1998). Plant-parasitic nematodes have negative effects on edible crops, ornamentals, turf, and forest trees. There are over 4,100 species of plant-parasitic nematodes that cause an estimated $US 80 billion loss per year worldwide (Decraemer and Hunt, 2006; Nicol *et al*., 2011). *Aphelenchoides fragariae* is a species of foliar nematode that is an increasingly widespread pathogen of ornamental crops with a wide host range, attacking more than 250 plant species in 47 plant families (Franklin, 1965). The wide host range is the reason why controlling this nematode is difficult (Dropkin, 1980). This species tends to feed inside tender leaves of plants and negatively impact ornamental plants growing in greenhouses, nurseries, and in the landscape (Dunn, 2005; Ritzema-Bos, 1891).

Aphelenchoides fragariae has a variety of common names, including bud and leaf nematode, fern nematode, strawberry spring dwarf nematode, and strawberry crimp nematode (CABI, 2017). The nematodes are commonly associated with temperate regions, being reported across the United States and in various countries around the world. *A. fragariae* is found on a diverse range of plants including ferns and bedding plants (Kohl, 2011). They are known to feed ecto- and endoparasitically on the above ground parts of plants, but can also be considered mycetophagous (Winslow, 1960; Hunt, 1993). In order to infect the host plant leaves, *A. fragariae* migrate up plant stems or are transported by water onto the leaf and enter through the stomata. Moisture such as dew, rain, and overhead irrigation favor nematode infection and aid in dispersal (Wallace, 1959; Winslow, 1960).

Aphelenchoides fragariae was first described by E. A. Ormerod when he sent infected strawberry plants to Ritzema-Bos in England. The visible symptoms on the plants included stunting and a deformity on the crown and lateral branches similar to a cauliflower, thus describing it as "cauliflower disease." The infected sample was sent in 1890, leading to Ritzema-Bos naming the nematodes that cause the disease *A. fragariae* the following year in 1891 (Goodey, 1933).

The common name strawberry crimp nematode was given to *A. fragariae* because of its association with damage among California strawberries. When seen on strawberries, foliar

nematodes may cause symptoms such as curled or crinkled leaves (crimp), deformed buds and flowers, or a reduction in flowering and in fruit set. The name strawberry crimp nematode should not be frequently used because it may be misleading as the nematode affects many other hosts and causes various symptoms (UC IPM, n.d.). *A. fragariae* is associated with the reduction of strawberry yields around the world, including up to a 60% yield reduction in Ireland and is involved in strawberry decline in France (Kohl, 2011).

Aphelenchoides spp. are obligate parasites, feeding on the above-ground parts of plants. They enter and exit a leaf through the stomata on either side of a leaf or penetrate the epidermis on the underside of a leaf. Water is a major factor in the movement and dispersal of foliar nematodes (Strümpel, 1967; Wallace, 1959). Moisture such as overhead irrigation and rainfall allow nematodes to migrate from one plant to another and transfer from leaf to leaf (Lehman, 1996). Peak populations of *A. fragariae* were observed from March to May and November to January in Poland, being influenced by factors like moisture and temperature. These nematodes are able to survive in a dormant state in desiccated leaves for up to 46 days and can survive in the soil without a host for up to 3 months (Stewart, 1921; Kohl, 2011). Therefore, removal of infected plant debris has been suggested as a good sanitation practice to manage this nematode.

After migrating through water films from plant stems to the leaves, *A. fragariae* penetrate through the leaf's stomata. Inside the leaves, nematodes are able to migrate, feed, molt, and lay their eggs throughout the leaf (Hesling and Wallace, 1961). Female nematodes will lay 25-32 eggs. These eggs will hatch within 3-4 days and mature within 6-12 days. The average life cycle taking approximately 2 weeks (Strumpel, 1967). *A. fragariae*, similar to typical plant-parasitic nematodes, goes through four different juvenile stages and four different molts in order to become either an adult male or female. The nematodes reproduce amphimictically (Cayrol and Dalmasso, 1975). The nematodes' destructive feeding causes the distinct symptomatic features such as the interveinal chlorosis and necrosis of the leaf, which may lead to defoliation. Sufficient moisture may allow nematodes to migrate from leaf to leaf and severely damage the plant. Juvenile and adult nematodes are able to survive the winter in desiccated leaves for some period of time. These dead leaves may also act as transportation for the nematodes when moved or blown around, helping to disperse the nematodes to new host plants (Hesling and Wallace, 1961).

The most recognizable field symptom of foliar nematodes are the interveinal lesions on the foliage (Fig. 1.1). Although, these lesions may also be caused by several bacterial diseases that consist of similarly confined vein patterns. In order to differentiate between the two, simple laboratory tests such as a Baermann funnel assay can be conducted to confirm the presences of nematodes. The plant cells that the nematodes feed on lose color, turn brown and eventually die. These symptoms are visible in the plant's leaves, the darkest lesions usually being desiccated.

Figure 1.1. Foliar nematode (*Aphelenchoides fragariae*) feeding damage causing interveinal lesions on the foliage of palapalai (*Microlepia strigosa*).

These lesions are interveinal due to the fact that the nematodes are so delicate they're unable to move easily through tough leaf tissue. After the nematodes have fed on one interveinal area, they exit the leaf through the stomata in order to enter a new area of healthy plant tissue on which to feed. When two plants come into direct contact, the nematodes with the help of free moisture, transfer and are easily dispersed from one plant to another (Dunn, 2005).

1.2. Ornamental Plants Important for Hawai‘i’s Ornamental and Landscape Industry

The plants used in this thesis research include *Anthurium adraeanum*, *Frangipani*, *Raphiolepis indica*, *Phalaenopsis*, *Hibiscus*, and *Microlepia strigosa*. These plant species were chosen because of their high value and popularity as ornamental plants in Hawai‘i.

Hibiscus sp.is a perennial, woody ornamental popular in home gardens and landscapes (Gettys, 2012). The hibiscus flowers have wide funnels, vary in color and have crimson centers. Hibiscus plants that are perennials will typically reach mature height within 2 to 3 years with sufficient moisture (Russ, 2004). These plants thrive in full sun and in loamy soil that is kept moist during the summer blooming period, although, it grows well in wet soil conditions and can survive in poorly drained sites (West and Preece, 2004). *Hibiscus* is known to be affected by scales and nematodes (Knight *et al*., 1997).

Fern plants have been reported to be hosts of foliar nematodes, more specifically palapalai (*Microlepia strigosa*) is a known host for *A. fragraie* (Kohl, 2011). Palapalai is of the Dennstaedtiaceae and indigenous to Hawai‘i. This fern thrives in shady and moist habitats (Anonymous, 2019). The palapalai fern has rich green foliage and is often used to make lei for hula competitions (Cook, 2013). In 1997, the estimated wholesale value of cut fern fronds in the United States exceeded $60 million per year (Uchida and Kadooka, 1997).

Anthurium (*Anthurium andraeanum*) is second in trade value among tropical cut flowers in Hawai‘i, after the orchid. Anthurium belongs to the Araceae and is native to Colombia and Ecuador (Chen *et al*., 2003; Dufour and Guerin 2003; Govaerts and Frodin, 2002). It is a slow-growing, monocotyledonous perennial that thrives in shady, warm, and humid climates. The main ornamental characteristics of this flower is its brightly colored spathe leaf and protruding inflorescence, which is called a spadix (Collette *et al*., 2004). Anthuriums produce long lasting flowers year-round, each leaf axil producing one flower. The plant keeps the sequence of leaf, flower, and new leaf throughout its life cycle, however leaf emergence differs with changing environmental conditions. The summer months are favorable for plant growth, whereas the lower temperatures and less light of winter months are less favorable to plant growth (Higaki *et al*., 1995). In Hawai‘i, *A. fragariae* is known to cause foliar blight of anthurium, which can be deadly in young plants (Hunter *et al*., 1974).

Plumeria (*Plumeria sp.*) are native to tropical Americas and belongs to the Apocynaceae (Criley, 2005; Zahid *et al*., 2010). They grow as small flowering trees and are commonly used as an ornamental, for shade, or in plant groupings (Nelson, 2009). With a broad, round-headed canopy, the tree is usually as wide as it is tall. Some types of plumeria shed their leaves annually, shedding leaves during the winter and having new leaf emergence during or following the spring. Flowers of various colors including white, red, pink, yellow, and colors in between, bloom from March to October (Criley, 2005). The plumeria flowers have a sweet aroma and are primarily used for making leis. Their average annual sales value in the state of Hawai‘i from 2004 to 2009 was an estimated $505,000 (Nelson, 2009).

Indian Hawthorn (*Rhaphiolepis indica*) belongs to the *Rosaceae* and is native to southern China and Japan. It has been cultivated across the U.S. and is often used as a foundation shrub for landscapes (Heartsill, 2017). It is generally a low-growing, evergreen, shrub that grows clusters of small flowers (Russ, 2016). Indian Hawthorn grows best on well-drained soils and it tolerates drought conditions and salinity well. Plants with less than 6 hours of sun are more susceptible to leaf spot diseases and may start to defoliate, but good air circulation could help to deter that. Indian Hawthorn is known to be affected by soil nematodes and scales (Gilman, 1999).

The Phalaenopsis orchid (*Phalaenopsis sp.*) is a member of the Orchidaceae and is native throughout southeast Asia (Yim, 2014; Dressler, 1993). They are monopodial consisting of a short upright stem and large fleshy leaves that resemble the moth they’re named after (Yim, 2014). Phalaenopsis orchids have a diverse distribution throughout Asia, thus the floral seasons may vary for individual phalaenopsis species. Most of the species have a single flowering time per year in response to the changing of seasons, but there are a few species that bloom twice a year or at irregular times (Ikedo, n.d.). In 2000, the wholesale value of potted and blooming moth orchids was over $100 million (USDA, 2001). Out of all the orchid species, the moth orchid is the most popular for potted plants (Wang, 2002).

1.3. Current Control Measures of Foliar Nematodes

Previously, chemical treatments using active ingredients such as oxamyl and parathion were observed to be effective against foliar nematodes (Kohl, 2011). Due to environmental concerns and their toxicity, these chemicals are no longer available for foliar nematode control (Kohl, 2011). In 1997, methyl bromide was effectively used against soil fungi, soil-based

nematodes, insects and weeds, making it the fourth most commonly used pesticide in the United States at the time. Although successful in killing these pathogens, it also killed the plant as well. Methyl bromide is considered an ozone-depleting substance and was regulated by the Montreal Protocol to eliminate the use of it in developed countries by 2005 (Chitwood, 2003).

Modern chemical control for foliar nematodes is now more environmentally friendly, although variable results have been reported (Kohl, 2011). Some chemical treatments were observed to be unsuccessful in killing nematodes in infected leaves despite being able to kill nematodes in water suspensions. Chlorfenapyr, typically used as a miticide, is currently labeled to control foliar nematodes on ornamentals in the greenhouse. Against thin-leaved plants such as anemone, this chemical proved to reduce foliar nematode populations, unlike crops with thicker leaves such as lantana where it seemed to be less effective (Kohl, 2011).

A total of three new nematicides will be tested throughout this thesis project. ESP 715 was developed by Bayer. MBI 304 and 305, two microbial based products, are developed by Marrone Bio Innovations. The active ingredient of ESP 715 (Bayer, Leverkusen, Germany) is fluopyram, which is also a SDHI (succinate dehydrogenase inhibitor) fungicide (Crow *et al*., 2017). The succinate dehydrogenase complex is the smallest complex in the respiratory chain. As a fungicide, fluopyram inhibits fungal respiration (Avenot and Michailides, 2010). In the case of nematodes, fluopyrams mode of action is to block a nematodes cellular respiration and restrict their ability to produce energy. Nematodes affected by this active ingredient will be drained of energy, straighten, become immobile, eventually stop feeding, and die (Backed by Bayer, 2017). Multiple field trials using the active ingredient fluopyram have been conducted on turf and golf courses in the United States (Crow *et al*., 2017). Researchers at the University of Florida found that fluopyram reduced sting nematode population densities and treated turf response positively 6 to 8 months or longer after one single application. Trials using fluopyram against *Anguina pacificae*, the Pacific shoot-gall nematode, were conducted by J. Baird, Marco Schiavon, M. Mundo and J. O. Becker at the University of California, Riverside. One to two applications of extremely low rates of fluopyram provided season-long protection on putting greens against high populations of *A. pacificae.* These trials were conducted on golf courses located from the coastal Monterey Peninsula to the San Francisco Bay Area (Crow *et al*., 2017). MBI 304 is a bionematicide modeled after a *Chromobacterium* spp. based biopesticide, Grandevo (Marrone Bio Innovations, Davis,

California). The *Chromobacterium* spp. strain extract fends off, stops feeding of, reduces reproduction, and induces mortality in sucking and chewing insects, flies and mites thus prevents the development of damaging populations of these pests. This product has not yet been tested on nematodes (Marrone Bio Innovations Annual Report, 2015). The *Chromobacterium* extract from MBI 304 has been previously used in a biopesticide, Grandevo for nuts, fruits, vegetables, turf and ornamentals (Marrone Bio Innovations, 2017). MBI 305 is a bionematicide also known as Majestene (Marrone Bio Innovations, Davis, California), which was approved by the EPA in 2014. It is based on an extract of *Burkholderia* spp. strain A396 and modeled after Venerate. The *Burkholderia* spp. strain extract degrades exoskeletons and interferes with molting in insects and mites. This product has previously been reported to work against soybean cyst, root-knot, lesion, stunt, reniform, lance and burrowing nematodes (Marrone Bio Innovations Annual Report, 2015). Anecdotal observation suggested that Majestene has effects against eggs, juveniles and adult lesion, root-knot, dagger, stunt, reniform and soybean cyst nematodes. Majestene reduced galling in potatoes, strawberries, tomatoes, peppers, cucurbits, corn, and onions (Marrone Bio Innovations, 2017).

1.4. Research Objectives

The overall goal for this thesis research is to identify new, reduced-risk nematicides against foliar nematodes for use on certain ornamental plants. Specific objectives are to:

1) Determine the efficacy of ESP 715, MBI 304 and Majestene for managing foliar nematodes on palapalai fern, and

2) Determine if ESP 715, MBI 304 and Majestene are phytotoxic to ornamental plants commonly used in Hawai‘i landscape industry.

Chapter 2. Nematicide Efficacy Against Foliar Nematodes on Ornamental Plants

2.1. Introduction

Aphelenchoides spp. are abundant throughout the United States, Canada and Europe and are known to cause serious damage on many ornamentals including ferns in nursery and landscape settings (Grewal and Jagdale, 2001; Heinlein, 1982; Johnson and Gill, 1975; Richardson and Grewal, 1993; Southey, 1993). Infected ornamental plants suffer visual disfigurement and reduced growth. Furthermore, returned shipments of nematode-infected plants cause millions of dollars in loss of revenue for nurseries. These losses demonstrate the need for additional foliar nematode control options.

Previously, hot water treatments have been used to control *A. fragariae* in infected hosta and fern plants (Jagedale and Grewal, 2004). A 90°C water drench was effective as a preventative treatment in autumn or spring in reducing foliar nematode infection of hosta without affecting the plant vigor. As for hot water treatments on fern, a different protocol may be needed. The hot water treatments did not reduce nematode infection/population but there were also no damaging effects observed on plant growth (Jagedale and Grewal, 2004). Hot water drenches may be further researched and possibly used as an alternative control method for foliar nematodes on fern. Until that time, a need for chemical control methods or other control methods are needed for foliar nematodes for homeowners and nursery managers alike. Although there are various chemicals that have suppressed foliar nematode (Jagdale and Grewal, 2002; LaMondia, 1999), the U.S. Environmental Protection agency (EPA) has banned the use of these products due to concerns regarding the environmental pollution and risks to human health (Nixon, 2001; Schulze, 2001). The objective of this experiment was to determine the efficacy of ESP 715, MBI 304 and Majestene for managing foliar nematodes on palapalai fern.

2.2. Materials and Methods

Nematode Inoculum

Foliar nematodes were collected from infected fern samples provided by a local nursery located on the windward side of the island of O‘ahu. Symptomatic leaves were placed in Baermann funnels at room temperature for 2 days. Nematodes were harvested from the funnels by draining into centrifuge tubes and filled with water to 50 ml. After at least 24 hours, the top 45 ml was

pipetted out of the tube leaving 5 ml of nematode solution. These nematodes were observed under a compound microscope and morphologically identified as *Aphelenchoides* sp. The nematodes were transferred onto sterile carrot disc cultures. Carrot disc cultures were established and maintained using the protocol of Coyne *et al*. (2014). Store bought carrots were used and all tools were autoclaved before use. The cultures were maintained in the dark at around 21-23°C. Nematodes were reinoculated onto new carrot discs every 5-7 weeks to further increase the population. Inoculation method comparisons were conducted on hibiscus and fern plants before starting the experiment to confirm that our inoculation technique was feasible. Leaves were randomly selected and wrapped with wet tissue paper. Inoculations were done using a pipette to place nematodes directly onto the damp tissue paper covering each leaf. Each individual leaf was inoculated with juvenile and adult nematodes and the whole plant was then covered with a black plastic bag for 72-96 hours in accordance to Walker *et al.* (1997). After allowing the nematode populations to establish for 6 weeks foliar nematode symptoms were visible. Symptomatic leaves were then removed, placed into Baermann funnels, and nematodes were then extracted proving the symptoms to be caused by the foliar nematodes.

Nematicide efficacy

Efficacy was determined for ESP 715, MBI 304 and Majestene against *A. fragariae* on palapalai fern, *Microlepia strigosa.* ESP and MBI products were evaluated in separate trials. The experiments consisted of 4 replications per treatment and included noninoculated negative and inoculated control plants, for a total of 24 plants. The experiment was conducted at Magoon Research Station in Mānoa. Plants were placed on benches in a shade house in a completely randomized design. Plants were watered manually according to their watering needs. ESP 715 was tested at 0.66 and 1.34 ml a.i/L. MBI 304 was tested at 4,793 mg/L and MBI 305 was tested at 20 ml/L. Controls consisted of untreated noninoculated and untreated inoculated plants. Five to 6 weeks after nematode inoculation, all chemicals were applied using a hand-held sprayer. The sprayer was calibrated prior to use to allow for sufficient canopy coverage using 6 ml per plant. One chemical application was made at the beginning of the experiment as a curative treatment. Nematode symptom evaluations were conducted weekly through 6 weeks starting 1 week after the initial application. Weekly severity ratings were evaluated on a 1-5 scale, the higher the number the more visual nematode symptoms were observed. Each week, two independent evaluations were

made, and the scores combined. The ESP trial was conducted from December 2018 to February 2019 and the MBI trial was conducted from February 2019 to April 2019.

Final nematode populations were assessed from the foliage. At the end of the ESP trial, all symptomatic leaves were collected and placed in Baermann funnels for final nematode population assessments. At the end of the MBI trial, all fronds from each plant were collected 10 weeks after the experiment and placed in Baermann funnels for final nematode assessments. Fronds were placed in Baermann funnels, nematodes extracted and counted. All fronds from each plant in the MBI trial were collected 10 weeks after the treatment. Funnels were drained into centrifuge tubes and filled with water to 50 ml. After at least 24 hours the top 45 ml was pipetted out, leaving 5 ml of nematode solution. 6 ml of boiling water was then added to each solution to relax the nematodes and final nematode counts were assessed.

Initial evaluations were conducted the same day as treatment applications and final evaluations were conducted when fronds were collected for nematode assay. Weekly plant evaluations and symptom ratings were recorded through 6 weeks starting 7 days after the initial application. Two independent ratings were made on damage. Data were taken on the overall nematode damage (%) on an individual plant. Average damage was recorded from only the symptomatic leaves of each plant. Damage was rated on a 0-5 scale as: 0 = 0%, 1 = 1-20%, 2 = 21-40%, 3 = 41-60%, 4 = 61-80%, and 5 = 81-100% visual nematode damage. Plant height, width, and weight were recorded at initial and final evaluations.

Weather

Daily weather conditions were obtained from www.wunderground.com. Data were collected on low, high and average temperatures, and average precipitation in Honolulu, Hawai‘i. Typical temperatures and precipitation for the duration of the ESP715 trial (December 2019 to April 2019) were high 28.6°C, low 18.8°C, average 24.2°C, and average precipitation 0.074 cm. For the MBI 304 and Majestene trials (January 2019 to April 2019) high 29.7°C, low 17.1°C, average 24.0°C, and average precipitation 0.06 cm. High winds from a winter storm occurred from February 8, 2019 to February 10, 2019 (www.wunderground.com).

Statistical Analysis

The data were checked for normality. Plant growth and phytotoxicity data over the course of the experiment were analyzed using repeated measure analysis. Data were log [log10(x+1)] and square root [sqrt(x+1)] transformed when needed using SAS (SAS Inc., Cary, NC). Means were separated using Waller–Duncan k-ration (k=100) t-test. Only true means were presented.

2.3 Results

ESP Trial

In the ESP trial, the noninoculated plants remained nematode free at the end of the experiments. Similar nematode populations were observed in all treatments except the noninoculated control in the EPS trial (P=0.4993, Fig. 2.1). The greatest populations were recovered from those plants receiving no fluopyram and lowest in the 1.34 ml fluopyram/L rate (P<.0001, Fig. 2.1). Plant damage was greatest in the control (0 ml fluopyram/L rate). Plant damage decreased at the 0.66 and 1.34 fluopyram rates compared to the 0 rate (Fig. 2.2). No difference in height (P=0.3684), width (P=0.7498), or weight (P=0.1873) was detected during the 6-week period.

MBI Trial

MBI trials observed various results with severity. The inoculated control had the highest severity at 1.27 (p<0.0001, Figure 2.3) with the noninoculated control having the lowest average ratings for and severity at 0.42. The *Burkholderia* treatment had severity ratings that that were significantly lower than the inoculated control and higher than the noninoculated control. Severity ratings were significantly lower than the inoculated control and *Burkholderia* treatments but higher than the noninoculated control. There was no significant difference in height (p=0.9747) and weight (p=0.371) over the 6-week period. Width was observed to be significantly higher than all other treatments (p=0.008, Figure 2.4). A significant difference in nematode numbers per plant was seen in the MBI trial by the two control treatments (p=0.5458, Figure 2.5). The inoculated control had the highest number of nematodes compared to the noninoculated control having the lowest.

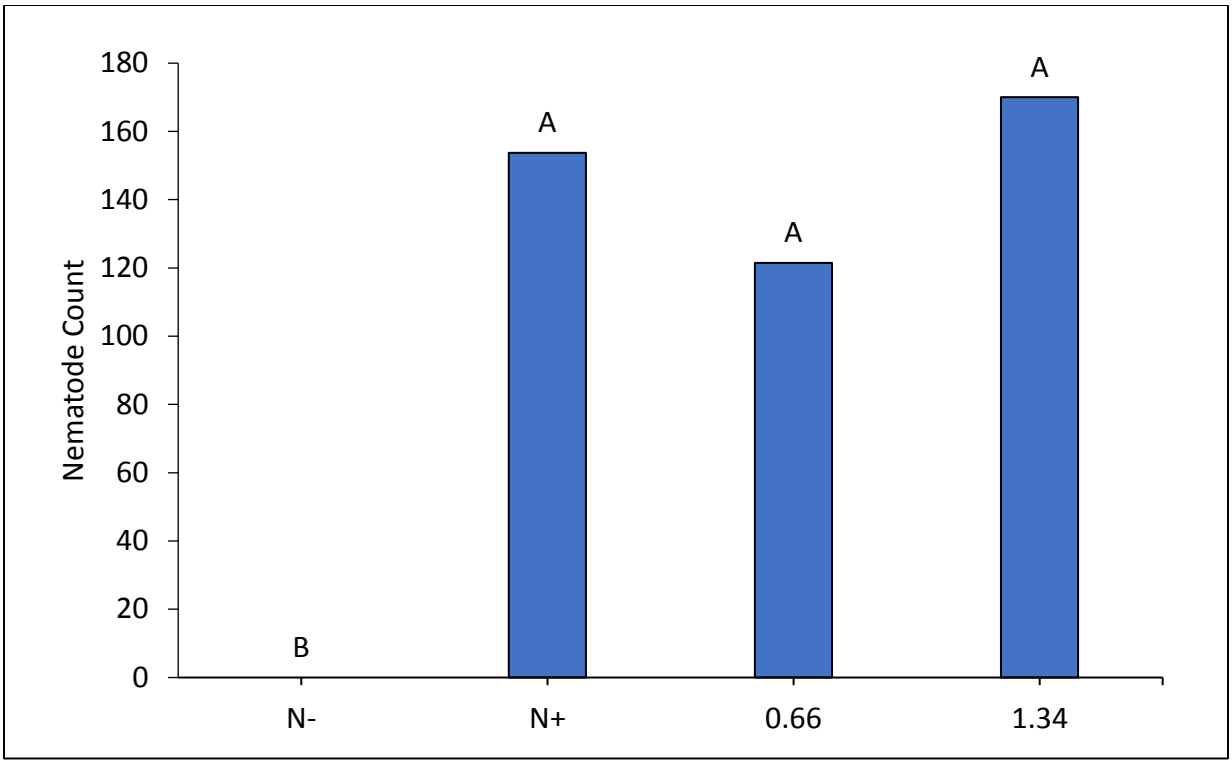

Figure 2.1. Population of *Aphelenchoides fragaraie* (N+ = inoculated, N- = nematode free) on *Microlepia strigosa* 6 weeks after treatment with fluopyram at 0, 0.66, or 1.34 ml ai./L. Columns (n= 12) topped by the same letters are not different accordin to Waller-Duncan *k*-ratio (*k*=100) *t*-test.

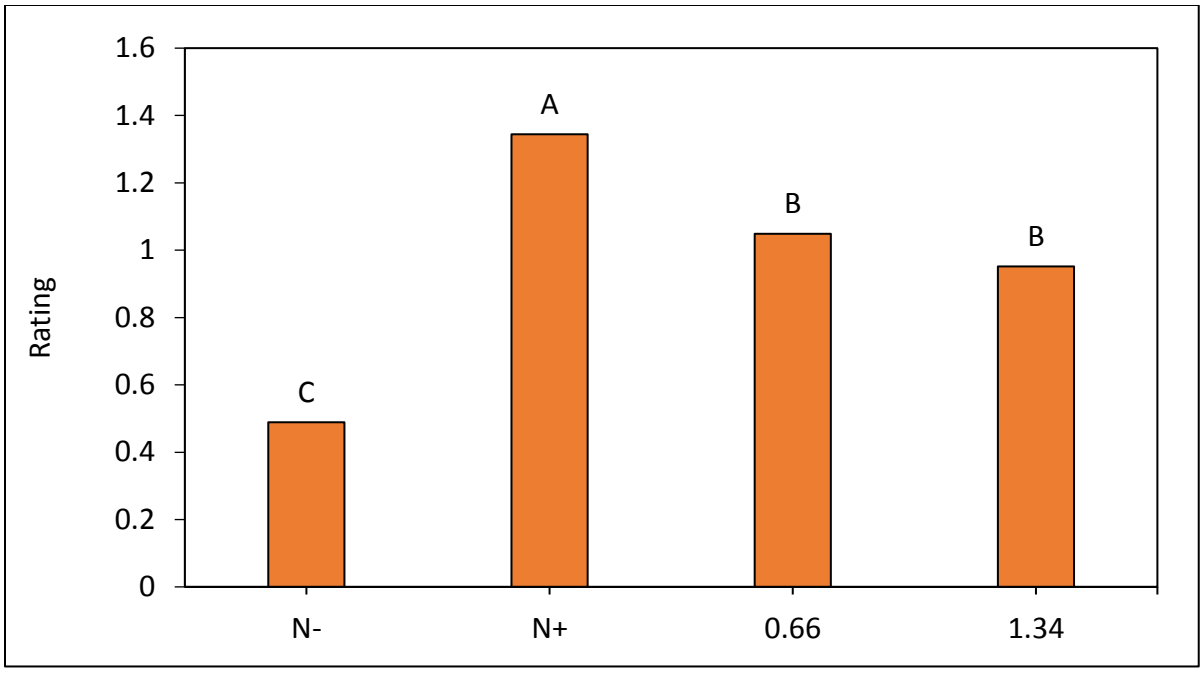

Figure 2.2. Ratings of foliar nematode severity on *Microlepia strigosa* inoculated with *Aphelenchoides fragaraie* (N+ = inoculated, N- = nematode free) and treated with fluopyram (0, 0.66, or 1.34 ml ai./L) 6 weeks after chemical treatment. Scale for percent of visible nematode symptoms on plant foliage: 0=0%, 1=1-20%, 2=21-40%, 3=41-60%, 4=61-80%, 5=81-100%.,. Columns (n=12) topped by the same letters are not different according to Waller-Duncan k-ratio (*k*=100) *t*-test.

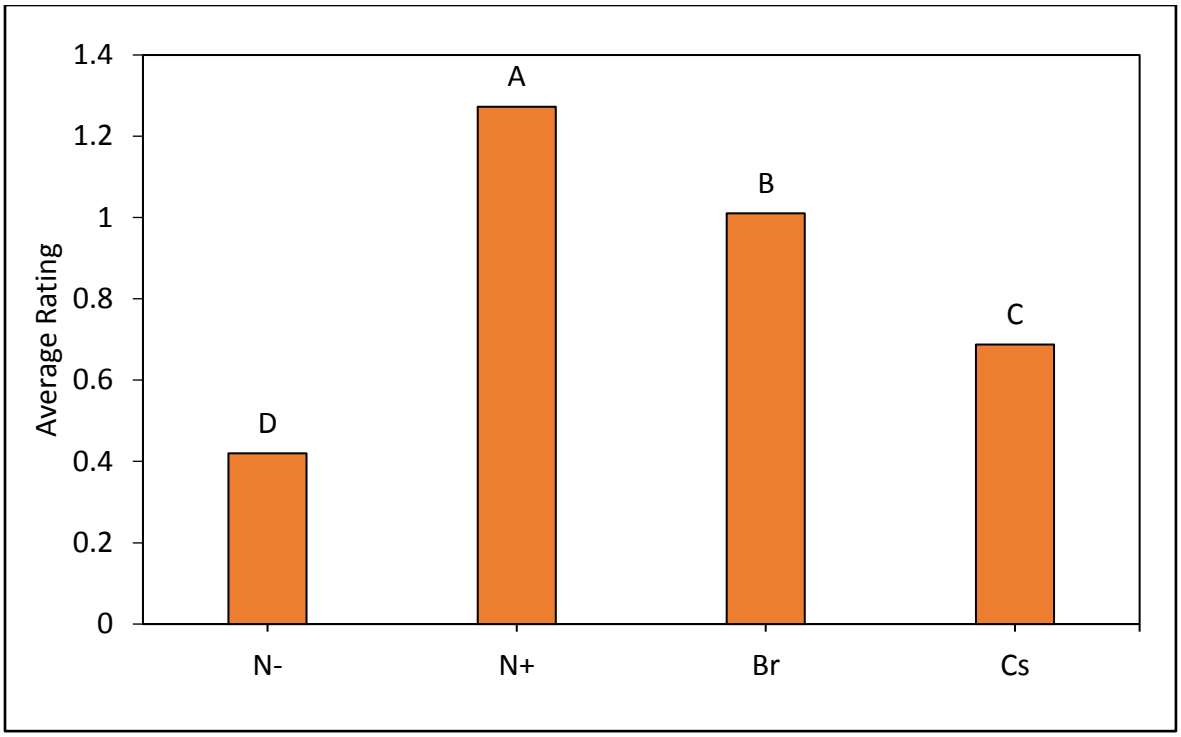

Figure 2.3. Ratings of foliar nematode severity on *Microlepia strigosa* inoculated with *Aphelenchoides fragaraie* (N+ = inoculated, N- = nematode free) and treated with *Burkholderia* (Br = Majestene) or *Chromobacterium* (Cs) 10 weeks after chemical treatment. Scale for percent of visible nematode damage on plant foliage: 0=0%, 1=1-20%, 2=21-40%, 3=41-60%, 4=61-80%, 5=81-100%. Columns (n=12) topped by the same letters are not different according to Waller-Duncan k-ratio (*k*=100) *t*-test.

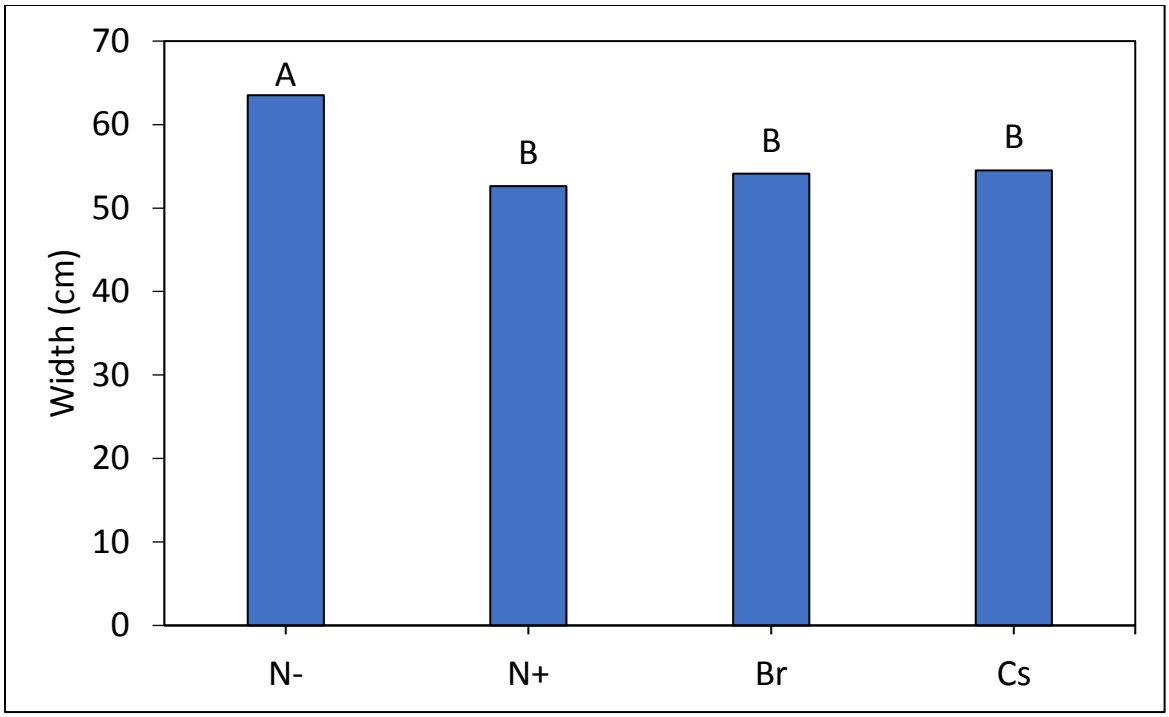

Figure 2.4. Plant canopy width (cm) of *Microlepia strigosa* inoculated with *Aphelenchoides fragaraie* (N+ = inoculated, N- = nematode free) and treated with *Burkholderia* (Br = Majestene) or *Chromobacterium* (Cs) 10 weeks after chemical treatment. Columns (n= 12) topped by the same letters are not different according to Waller-Duncan *k*-ratio (*k*=100) *t*-test.

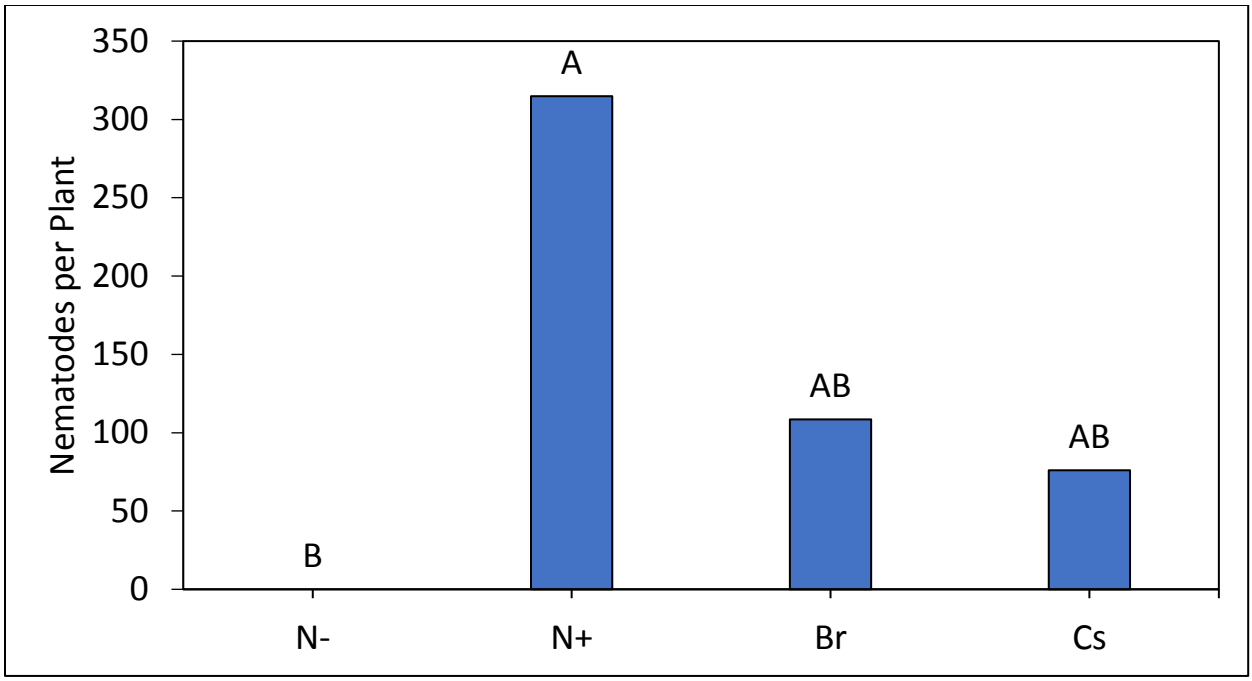

Figure 2.5. Population of *Aphelenchoides fragaraie* (N+ = inoculated, N- = nematode free) on *Microlepia strigosa* 10 weeks after treatment with *Burkholderia* (Br = Majestene) or *Chromobacterium* (Cs). Columns (n= 12) topped by the same letters are not different according to Waller-Duncan *k*-ratio (*k*=100) *t*-test.

2.4 Discussion

These new nematicides show promise in the management of foliar nematode in fern. Neither, fluopyram, *Burkholderia*, nor *Chromobacterium* adversely affected growth of palapalai. With fluopyram, increasing rates, lower damage was observed on the plants. Control treatments in the fluopyram trial having ratings higher than '0' suggest there were some environmental factors that affected the plant appearance. Even though nematode populations were similar among the fluopyram treatments, the similar numbers at the high rate could be attributed to better plant growth supporting greater numbers of nematodes. Both *Chromobacterium* and *Burkholderia* treatments reduced nematode populations. The greater plant growth associated with the MBI products may suggest that both *Chromobacterium* and *Burkholderia* may suppress foliar nematode and be an option for management commercial operations.

Chapter 3. Phytotoxicity Effects on Ornamental Plants Caused by New Nematicide

3.1 Introduction

Phytotoxicity, or plant injury, occurs when plants are exposed to certain chemicals or contaminants (Singh and Srivastava, 2015). Chemicals applied to control a pest may inadvertently cause damage to the plant and reduce its value. Both foliar sprays and soil drenching may cause injury to the leaves or flowers of plants. Phytotoxicity may be observed as different symptoms include leaf speckling, marginal necrosis or chlorosis, brown or yellow patches or spots, stunting, leaf distortion, or plant death (Getter, 2015). Phytotoxicity negatively affects the health and/or aesthetics of the plant and may result in reduced revenue.

Chemical products and their a.i. used in this experiment included ESP 715 with fluopyram, MBI 304 with *Chromobacterium* and Majestene with *Burkholderia*. Previously, fluopyram fungicide treatments saw phytotoxicity effects on soybean cotyledons. These effects resulted in discoloration on the tips of cotyledons, which can resemble disease or other abiotic stress (Wise *et al*, 2015). The *Chromobacterium* spp. strain extract was previously evaluated for phytotoxicity on marigolds. There was no phytotoxicity effects seen, with all ratings being 0% of phytotoxicity (Vafaie and Rydzak, 2015). Majestene was evaluated for phytotoxicity on a variety of crops as read on the specimen label, results were not presented (Marrone Bio Innovations, 2017).

The objective of this experiment was to determine the phytotoxicity of fluorpyram, and formulations of *Burkholderia* and *Chromobacterium*.

3.2. Materials and Methods

Experimental Design

The plants were obtained from commercial sources. Six plant species were evaluated: anthurium (*Anthurium andraeanum*), plumeria (*Plumeria* sp.), Indian hawthorn (*Rhaphiolepis indica*), orchid (*Phalaenopsis* sp.), hibiscus (*Hibiscus* sp.), and palapalai (*Microlepia strigosa*). The trial was conducted at Magoon Research Station. Plants were placed in a completely randomized design on benches in a shade house or in the open. Hibiscus, plumeria, and indian hawthorn plants were placed on open benches in full sun. Anthurium, phalaenopsis, and palapalai plants were placed on a bench inside the shade house. Plants were watered manually as needed. Hibiscus, phalaenopsis, and anthurium were evaluated between December 2017 to July 2018.

Indian hawthorn and plumeria were evaluated between March 2018 to October 2018. Palapalai was evaluated from January 2019 to July 2019.

Fluopyram, as the Bayer test formulation ESP 715, was evaluated at 0.33 ml/L, 0.66 ml/L, and 1.34 ml/L. *Burkholderia*, as MBI 304, and *Chromobacterium*, as Majestene were only evaluated on palapalai at rates of 20 ml/L and 4,793 mg/L, respectively. Fluopyram was tested only at 0.66 ml/L and 1.34 ml/L for palapalai. A control consisting of water was included with each plant species. ESP 715 and MBI 304 were applied using a hand-held sprayer, calibrated prior to use, to ensure canopy coverage. Based on the plant size, chemical applications were applied from 6 to 8 ml per plant. Three chemical applications were made 14 days apart. The experiment consisted of 10 replications of each chemical concentration tested for a total of 40 individual plants per plant species. Plumeria had 5 replications per each treatment and palapalai had 4 replications per treatment due to low plant availability.

Data Collection

Evaluations were conducted 7 days after each chemical application and then once a month for 5 months after the last application. For palapalai, monthly evaluations were made for 2 months. Initial evaluations were made before the first chemical application and final evaluations were made after the last observation day. For palapalai, initial data taken the same day as the first chemical application and final data were taken on the same day as the final day of observations. Plant weight was recorded for all plant species and were recorded at initial, final, and monthly evaluations. Before collecting plant weights, plants were saturated with water and allowed to naturally drain for 90 minutes before weighing. Initial, weekly and final evaluations of other plant growth parameters other than weight differed by the individual plants, being adjusted to the specific plant species growth habits and based on the IR-4 Project protocol.

Weekly plant evaluations were conducted 7 days after each chemical application. Phytotoxicity severity was evaluated on the affected leaves, rated on a scale from 0-5 (Reis *et al*., 2010). Plants were maintained for 5 months or 2 months for palapalai to evaluate growth defects. Monthly observations focused on stunting. Data were collected if there was any damage to the flowers and/or bud development.

Weather

Daily weather conditions during this experiment were obtained from www.wunderground.com. Data was taken on factors such as, the low, high and average temperature, percent of precipitation and percent of humidity in Honolulu, Hawai‘i. Typical temperatures and precipitation for the duration of the hibiscus, orchid, anthurium trial (December 2017-June 2018): high temperature 28.4°C, low 19.9°C, average daily temperature 24.2°C, average precipitation 4.34 centimeters. For plumeria and Indian hawthorn (March 2018-September 2018): high temperature 30.5°C, low 22.2°C, average daily temperature 26.4°C, average precipitation 2.65 centimeters. For palapalai ESP 715 trial (January 2019-April 2019): high temperature 29.7°C, low 17.1°C, average daily temperature 24.0°C, average precipitation 0.06 centimeters. For palapalai MBI 304 and Majestene trial (March 2019-June 2019): high temperature 31.4°C, low 19°C, average daily temperature 25.9°C, average precipitation 0.02 centimeters. Tropical storm warnings posted on 8/22/18 and 9/12/2018 may have impacted the trials but evaluations continued. Winter storm warning of high winds occurred from February 8, 2019 to February 10, 2019 may have impacted the palapalai ESP trial, but evaluations continued (www.wunderground.com).

Statistical Analysis

The data were checked for normality. Plant growth and phytotoxicity data over the course of the experiment were analyzed using repeated measure analysis. Data were log [log10(x+1)] and square root [sqrt(x+1)] transformed when needed using SAS (SAS Inc., Cary, NC). Means were separated using Waller–Duncan *k*-ratio (*k*=100) *t*-test. Only true means were presented.

3.3 Results

No visual foliar phytotoxicity symptoms were observed for fluopyram at any of the tested rates on hibiscus, orchid, anthurium, Indian hawthorn, or plumeria throughout the 26-week period.

Hibiscus: The control had significantly higher height than all other treatments, whereas chemically treated plants were consistently similar (*P*=0.006, Fig. 3.1). On the other hand, the control had the lowest weight and the chemical treatments resulted in significantly higher weight (*P*=0.0187, Fig. 3.2). There was no difference between any of the treatments in stem diameter measurements (*P*=0.1079).

Orchid: Spike length was highest in the control and descended significantly to 0.33 ml/L and 0.66 ml/L while 1.34 ml/L fell between 0.33 and 0.66 (P<.0001, Fig. 3.3). Weight was heaviest in the 1.34 ml/L treatment and significantly lower in all other treatments (P=0.0013, Fig. 3.4).

Anthurium: The number of flowers descended in order from 0.66 ml/L, 1.34 ml/L, 0.33 ml/L and control respectively (P<.0001, Fig. 3.5). Flower petiole lengths were longest in control and 0.33 ml/L. Those rates were significantly higher than 1.34 ml/L and 0.66 ml/L treatments, which was the rate with the shortest petiole lengths (P<.0001, Fig. 3.6). There was no difference in weight among any treatments (P=0.1976).

Indian Hawthorn: The 0.33 ml/L treatment had the biggest width, 1.34 ml/L with the lowest and the other two treatments falling in between (P<.0001, Fig. 3.7). Weight was lowest in 0.33 ml/L treatment and highest in 0.66 ml/L treatment, with the rest of the treatments falling in between (P=0.0013, Fig. 3.8). There were no differences in height among treatments in the 26-week period.

Plumeria: Height was highest in the control and 0.66 ml/L treatments, and lowest in 1.34 ml/L treatment (P=0.0308, Fig. 3.9). Stem diameter was biggest in 0.66 ml/L treatment, and lowest in the control and 0.33 ml/L treatment (P=0.0127, Fig. 3.10). There was no difference in weight among treatments throughout the 26-week period.

Palapalai: There were no differences in height (P=0.2951), width (P=0.6084) or weight (P=0.9392) among fluopyram treatments throughout the 14-week period. Palapalai was the only plant species to experience some visual foliar phytotoxicity symptoms, as early as the first observation. These symptoms were seen on all treatments except the control. Severity was similar in both chemical treatments which were significantly higher than the control (P<.0001, Fig. 3.11). In the *Chromobacterium* and *Burkholderia* treatments there were no differences in height (P=0.3054), width (P=0.242), or weight (P=0.2224) were observed in any of the treatments throughout the 14-week period. Phytotoxicity (P=0.3271) was not different between any of the treatments, the highest ratings observed being no higher than a 0.4 on the 0-5 phytotoxicity rating scale.

3.4 Discussion

No phytotoxicity effects were observed on five of the plant species used in the experiment. Hibiscus, phalaenopsis orchid, anthurium, Indian hawthorn and plumeria were tolerant to the rates of which fluopyram was applied. Differences in weight and other factors may be due to multiple

variables in the environment including: weather, physical damage, feeding pests and loss of soil overtime. Personal observations suggested that fluopyram could have acted as a possible snail deterrent when comparing chemical treatments and the control plants. After extensive snail feeding, control methods including snail bait and manual removal were used to suppress the snail population.

Palapalai having daintier foliage could be the reason that phytotoxicity effects were seen on this plant species. Although being the only plant species to experience phytotoxicity damage on the foliage, the damage ratings did not exceed a 3 in severity with averages being below a 1.4 in all damage ratings. There were no statistical differences between the control and bionematicide treatments, suggesting that they did not cause any phytotoxicity effects but may have been affected by outstanding variables such as weather, emerging disease and pests.

Symptomatic flowers were found on some hibiscus and orchid plants. Various symptoms including, flower deformity, discoloration and minor necrosis could be the result of outstanding variables and were not proven to be caused by the chemical itself. These variables included, physical damage from wind and/or falling over, emerging plant diseases or pests. Recovery was observed on majority of the plants by the end of the experiment, except what is believed to be *Botrytis cinerea* on orchid flowers which were present prior to experiments.

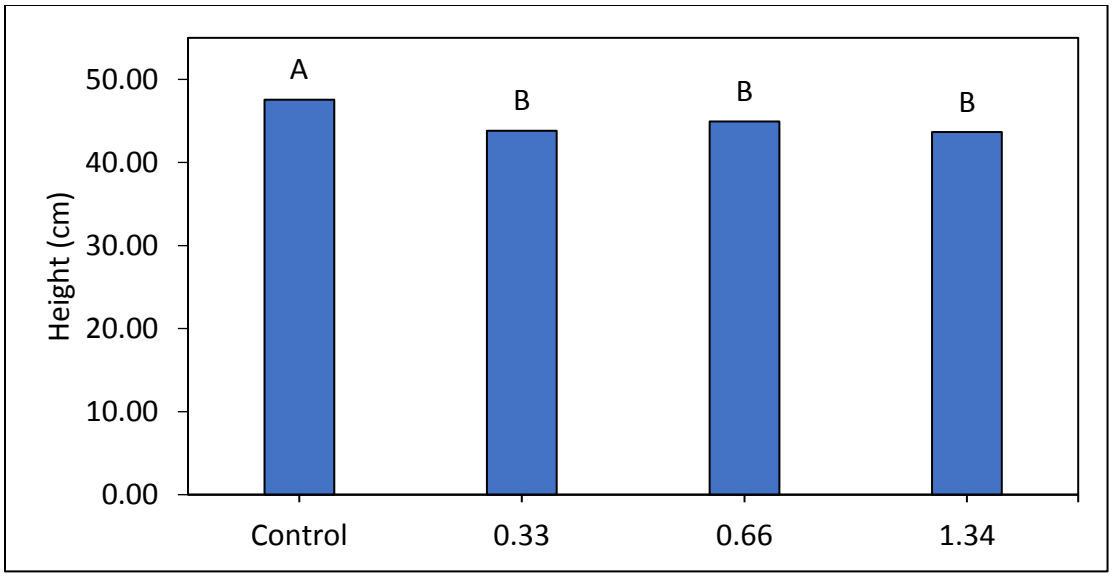

Figure 3.1. Effects of fluopyram at 0, 0.33, 0.66, or 1.34 ml fluopyram/L on hibiscus height over a 26-week period. Columns (n= 70) with the same letters are not different according to Waller-Duncan *k*-ratio (*k*=100) *t*-test.

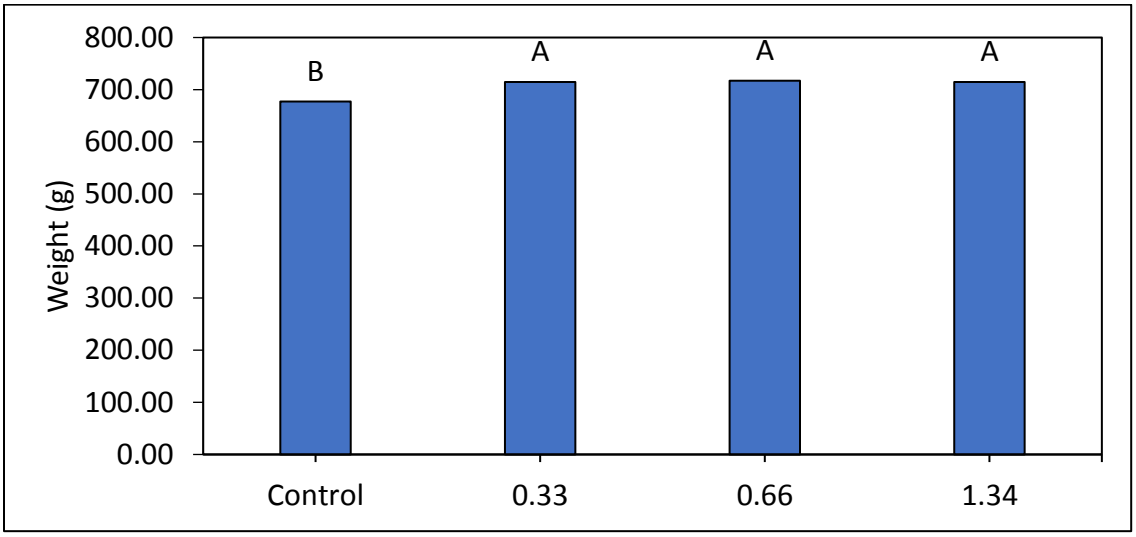

Figure 3.2. Effects of fluopyram at 0, 0.33, 0.66, or 1.34 ml fluopyram /L on hibiscus weight over a 26-week period. Columns (n= 70) with the same letters are not different according to Waller-Duncan *k*-ratio (*k*=100) *t*-test.

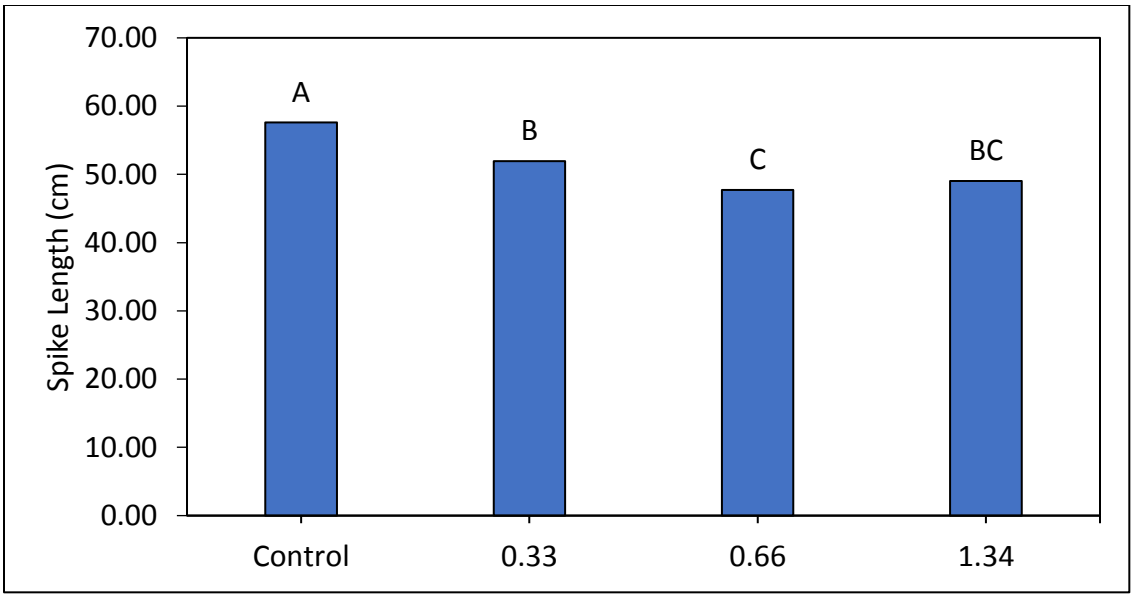

Figure 3.3. Effects of fluopyram at 0, 0.33, 0.66, or 1.34 ml fluopyram /L on orchid spike length over a 26-week period. Columns (n= 70) with the same letters are not different according to Waller-Duncan *k*-ratio (*k*=100) *t*-test.

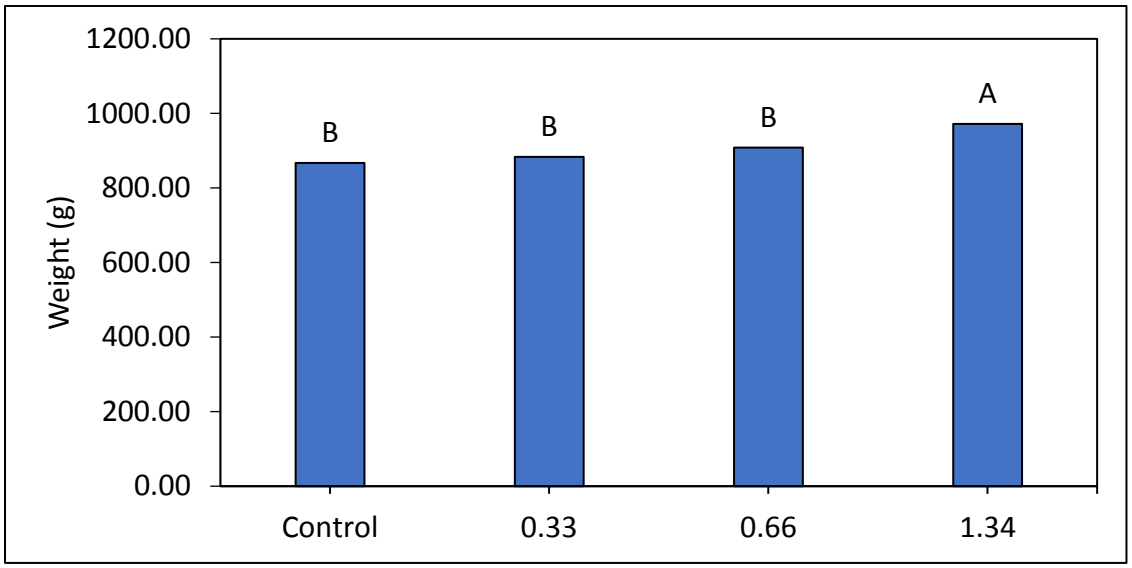

Figure 3.4. Effects of fluopyram at 0, 0.33, 0.66, or 1.34 ml fluopyram /L on orchid weight over a 26-week period. Columns (n= 70) followed by the same letters are not different according to Waller-Duncan k-ratio (k=100) t-test.

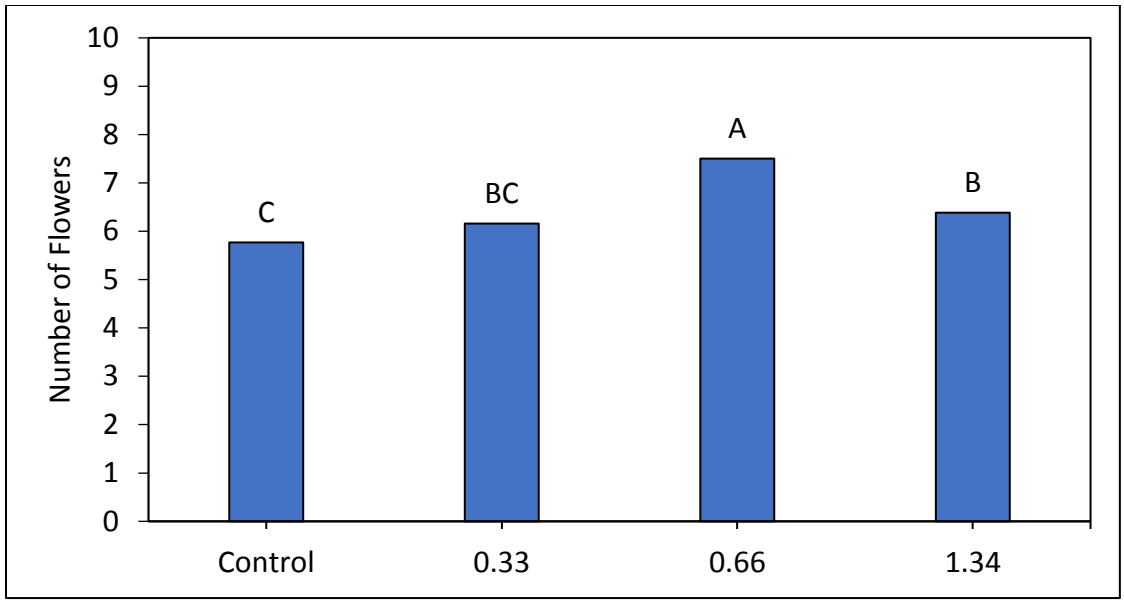

Figure 3.5. Effects of fluopyram at 0, 0.33, 0.66, or 1.34 ml fluopyram /L on anthurium number of flowers over a 26-week period. Columns (n= 70) followed by the same letters are not different according to Waller-Duncan k-ratio (k=100) t-test.

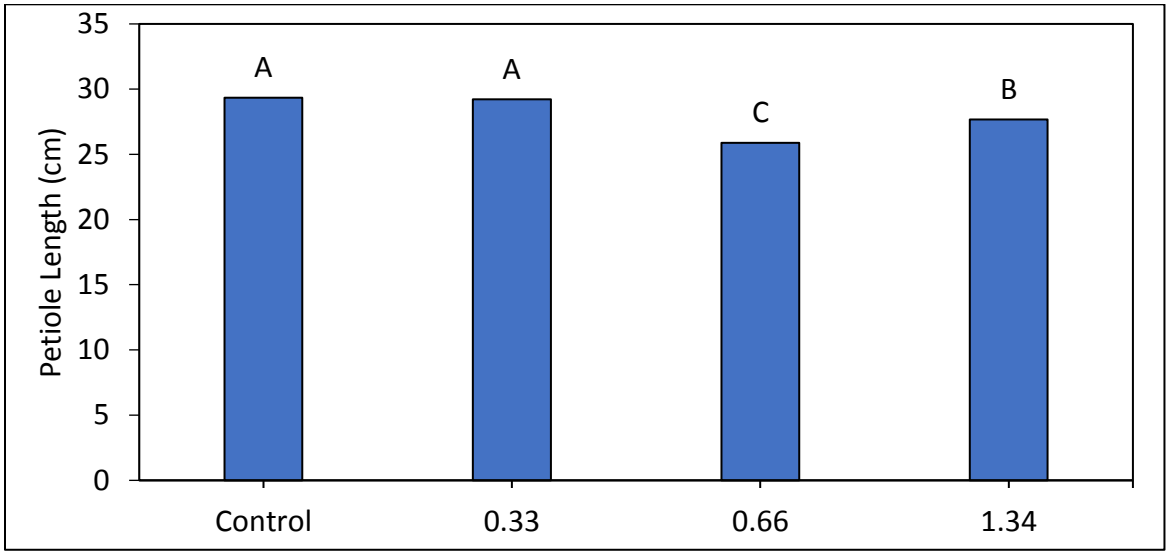

Figure 3.6. Effects of fluopyram at 0, 0.33, 0.66, or 1.34 ml fluopyram /L on anthurium petiole length over a 26-week period. Columns (n= 70) followed by the same letters are not different according to Waller-Duncan k-ratio (k=100) t-test.

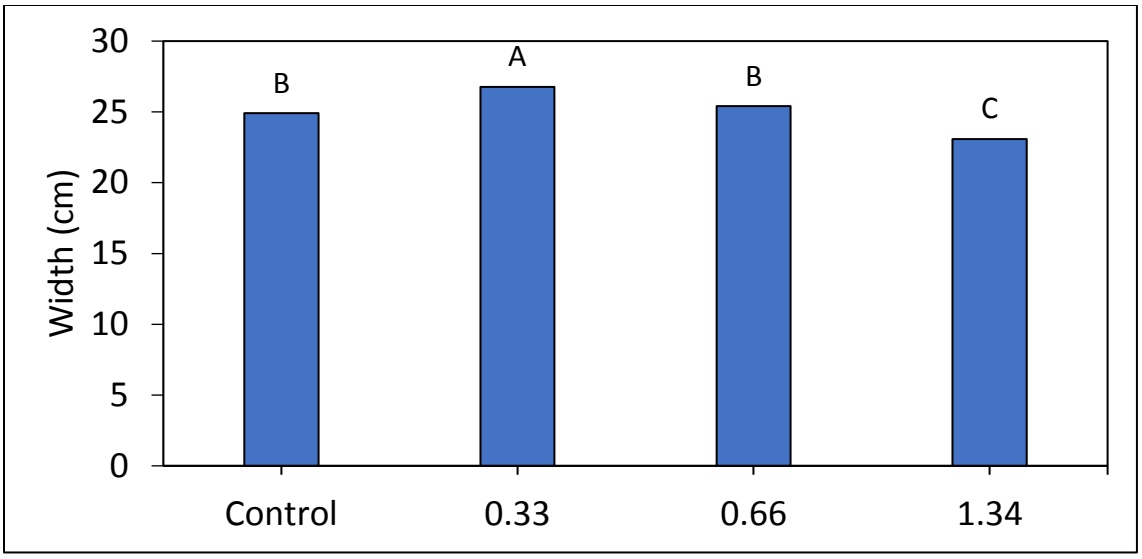

Figure 3.7. Effects of fluopyram at 0, 0.33, 0.66, or 1.34 ml fluopyram /L on indian hawthorn width over a 26-week period. Columns (n= 70) followed by the same letters are not different according to Waller-Duncan k-ratio (k=100) t-test.

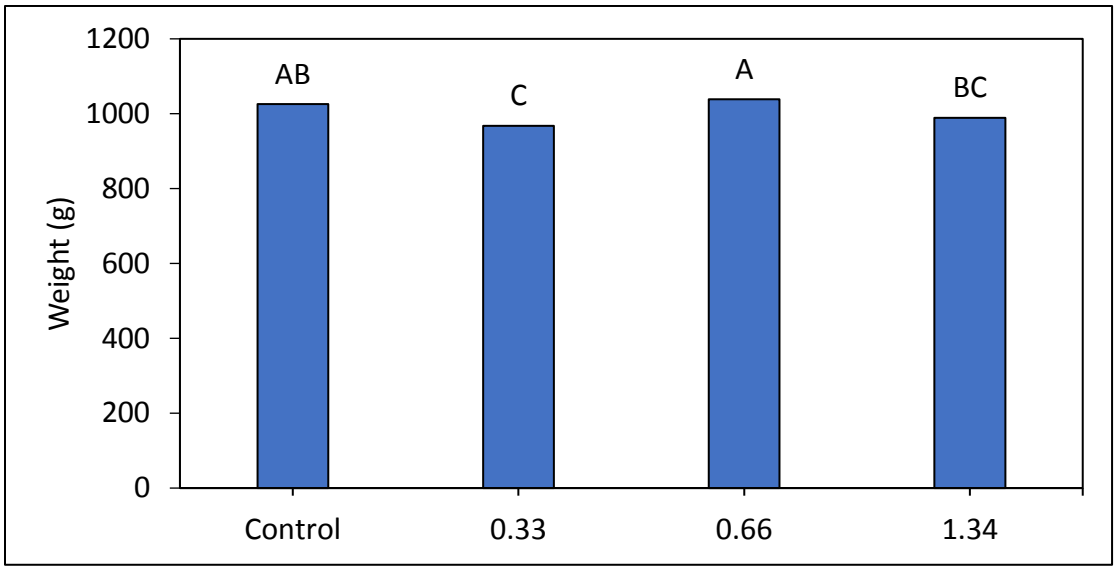

Figure 3.8. Effects of fluopyram at 0, 0.33, 0.66, or 1.34 ml fluopyram /L on indian hawthorn weight over a 26-week period. Columns (n= 70) followed by the same letters are not different according to Waller-Duncan k-ratio (k=100) t-test.

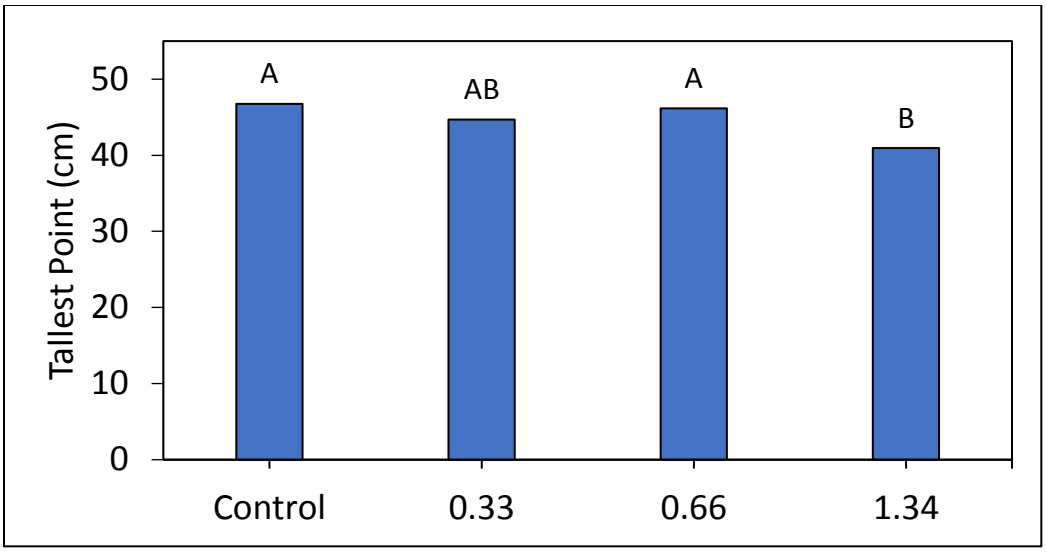

Figure 3.9. Effects of fluopyram at 0, 0.33, 0.66, or 1.34 ml fluopyram /L on plumeria height over a 26-week period. Columns (n= 35) followed by the same letters are not different according to Waller-Duncan k-ratio (k=100) t-test.

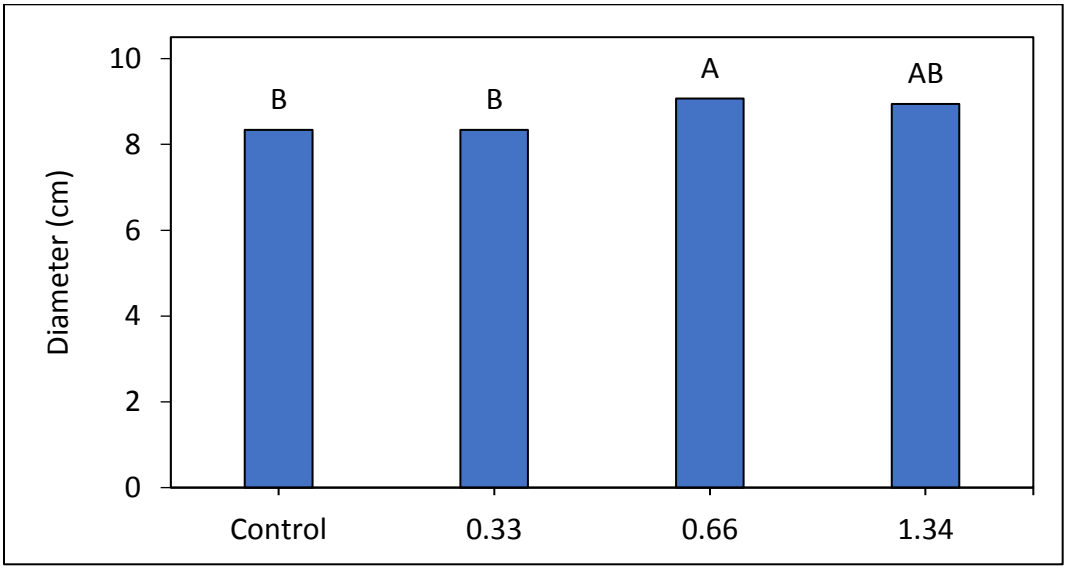

Figure 3.10. Effects of fluopyram at 0, 0.33, 0.66, or 1.34 ml fluopyram /L on plumeria stem diameter over a 26-week period. Columns (n= 35) followed by the same letters are not different according to Waller-Duncan k-ratio (k=100).

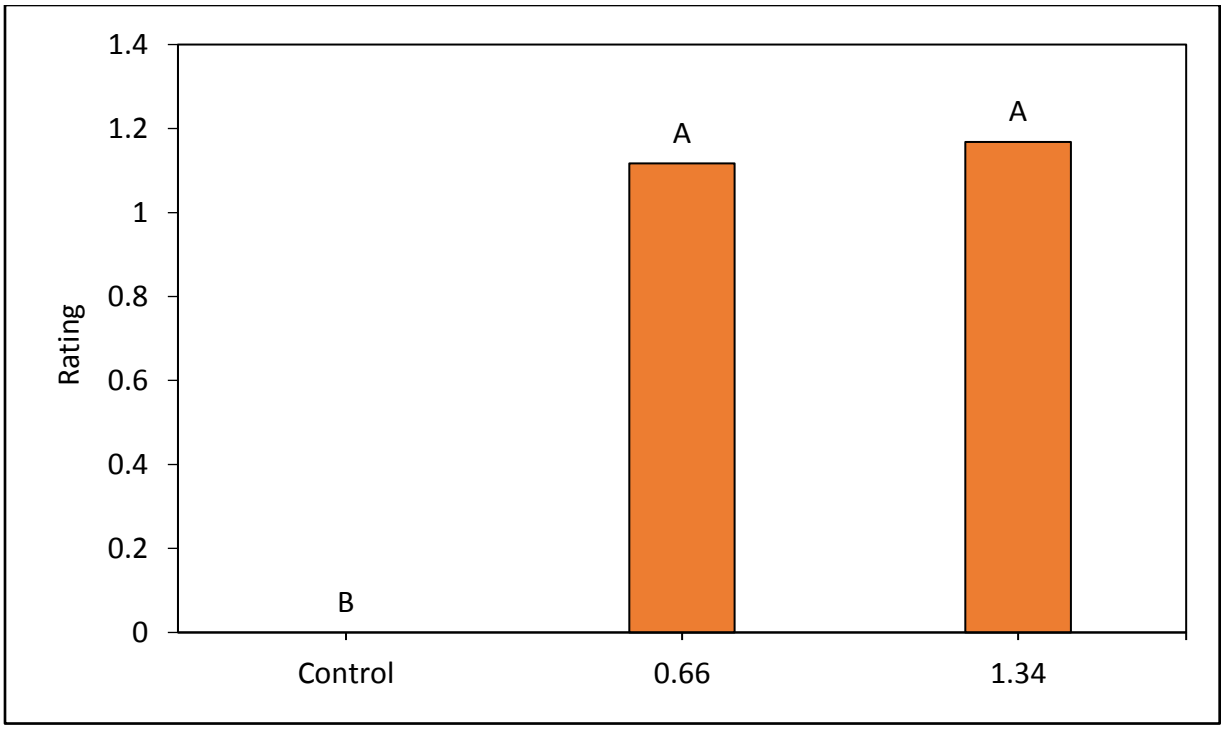

Figure 3.11. Fluopyram phytotoxicity severity ratings on *M. strigosa* for each treatment throughout the 14-week period. Rating scale for percent of visible nematode symptoms on plant foliage: 0=0%, 1=1-20%, 2=21-40%, 3=41-60%, 4=61-80%, 5=81-100%. Control: control, 0.66: 0.66 ml/L of fluopyram and 1.34: 1.34 ml/L of fluopyram. Columns (n= 24) followed by the same letters are not different according to Waller-Duncan k-ratio (k=100) t-test.

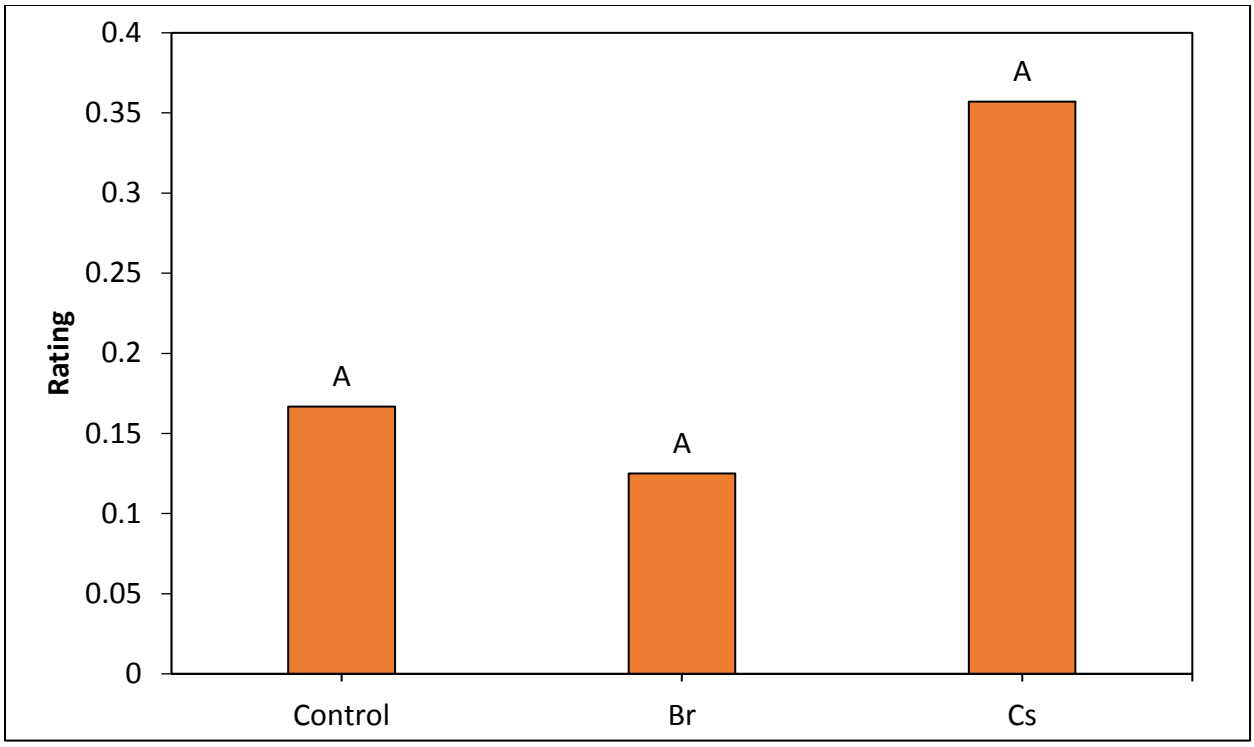

Figure 3.12. *Chromobacterium* and *Burkholderia* phytotoxicity severity ratings on *M. strigosa* for each treatment throughout the 14-week period. Rating scale for percent of visible nematode symptoms on plant foliage: 0=0%, 1=1-20%, 2=21-40%, 3=41-60%, 4=61-80%, 5=81-100%. Control: control, 0.66: 0.66 ml/L of fluopyram and 1.34: 1.34 ml/L of fluopyram. Columns (n= 24) followed by the same letters are not different according to Waller-Duncan k-ratio (k=100) t-test.

Chapter 4. Conclusions and Considerations

With limited control methods against foliar nematodes we are looking at newly developed nematicides that are effective and safe for the plants. There are no products currently on the market that are successful and/or registered to be used against foliar nematodes. Limitations like these make production especially difficult for fern growers.

Foliar applications of newly developed nematicide products from Marrone Bio Innovations, MBI 304 using a *Chromobacterium* spp. strain extract and Majestene using a *Burkholderia* spp. strain extract, suppressed 75.8% and 65.7% of foliar nematodes, respectively.

In addition, these bionematicides did not cause phytotoxicity on palapalai. Fluopyram did not suppress foliar nematodes, and it caused significant phytotoxicity on palapalai. No phytotoxicity by fluopyram was observed on all other plants tested (*Frangipani*, *R. indica*, *Hibiscus*, *Phalaenopsis*, and *A. adreanum*). Although some stunting of growth was observed, this did not affect the quality (marketability) of the plants. In particular, plant weight of hibiscus and orchid were increased by fluopyram, possibly due to snail hindrance of the chemical.

The use of bionematicides containing *Chromobacterium* and *Burkholderia* are suggested for the control of foliar nematodes on palapalai. Along with chemical applications, pruning of nematode symptomatic fronds may result in increased nematode suppression. Using proper irrigation techniques will also limit the spread of the nematodes. The combination of these methods is more likely to ensure the reduction of foliar nematodes within nurseries and greenhouses.

Future studies are needed to examine foliar nematode control on other plant species using fluopyram, *Chromobacterium* and *Burkholderia*. Biological products are good alternatives to traditional chemical products. The direction that some companies are going with these products, using biological active ingredients rather than chemicals, is a viable direction for the future of modern nematicides. Continuing to test various biological agents and/or products against pests will increase our chances of developing more environmentally friendly yet effective control methods against pests.

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