Nonchemical alternatives for pest management: Entomopathogenic nematodes and UV-C light.

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Non-chemical Alternatives for Pest Management: Entomopathogenic Nematodes and UV-C Light

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ABSTRACT

The primary objectives of this research are to determine effective biological and alternative control strategies of insect and disease pests to reduce harsh chemical use during greenhouse crop production and transport. This research includes two separate studies: 1) testing the practical viability of rearing and storing four species of entomopathogenic nematode (EPN), *Steinernema feltiae, Steinernema carpocapsae, Heterorhabditis bacteriophora, Heterorhabditis indica;* and, 2) the efficacy of UV-C radiation applied, pre-transport, as a preventative disease control strategy against *Botrytis cinerea.* A study was conducted to determine EPN infectious juvenile (IJ) counts and IJ viability after a six-day storage period. When all four species are compared, *S. feltiae* had a greater number of infectious juveniles emerge from the wax moth cadavers and *S. carpocapsae* had the least. All four species survived the six-day storage period but EPN infectious juvenile counts were significantly different among species. Our second study tested the efficacy of UV-C radiation as an alternative control to traditional fungicides to deactivate *B. cinerea* in vitro and to determine plant tolerance to UV-C. The crops tested were poinsettia (*Euphorbia pulcherrima*) and primula (*Primula vulgaris).* All the UV-C doses, 1.0, 2.8, 3.7 or 4 W/m^2, significantly decreased *B. cinerea* conidial germination in vitro and resulted in zero percent damage on poinsettia bracts. However, all UV-C doses during both replications caused minor damage, 15% or less, to primula flowers.

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ABSTRACT

Entomopathogenic nematodes (EPN) shows promise in being non-chemical and environmentally friendly solution for greenhouse pest and disease control. These can also be referred to as Biological Controls (Biocontrols). Entomopathogenic nematodes are used widely to control multiple greenhouse plant pests which include both *Lycoriella* spp., Fungus Gnats, and *Frankliniella* spp., Western Flower Thrips. However, there are challenges with EPN viability and storage from the manufacture to the greenhouse producer. We studied four EPN species, *Steinernema feltiae, Steinernema carpocapsae, Heterorhabditis bacteriophora, Heterorhabditis indica,* which were reared and stored to determine differences in production viability between species. Results show that the EPN species do not respond the same to storage and produce different amounts of infectious juveniles during rearing when conditions are the same. Separate from, but just as concerning as greenhouses plant pests are plant diseases. Ultraviolet radiation in the C spectra is known to be germicidal due to its narrow wavelengths. Because of this, UV-C has been shown to deactivate many different plant pathogens on contact and is being considered as a possible Biocontrol alternative to harsh traditional fungicides and bactericides. One disease that is known to contribute to the highest volume of annual crop losses is *Botrytis cinerea*. *Botrytis cinerea* is a plant disease that impacts floricultural crops to vegetables during propagation through the production supply chain to shipping and storage. We evaluated UV-C radiation at different doses, to determine if it could be used to replace a traditional fungicide before plants are shipped to reduce *B. cinerea* infection during transport. We found that UV-C successfully deactivated *B. cinerea* in vitro, but the viability of the application to plant tissue

before transport has yet to be proven successful as a practical method of reducing *B. cinerea* during transport.

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Chapter 1: Literature Review

Introduction

Biological Control is a non-chemical alternative that encompasses the use of predators, parasitoids and pathogens to control invertebrate pests along with the use of herbivores and pathogens to control weed pests and antagonistic microorganisms to control plant pathogens (Eilenberg et al., 2001). This form of integrated pest management (IPM) is one of the most common pest management trends in the North American and European greenhouse and agricultural markets. The strategy of controlling pests with other organisms has been in use, successfully, for decades on multiple crops. For example, Hajek et al. (2016), reports that classical biological control began in the 1800s and steadily increased in use until the late 1980s when scientists raised concern about possible unintended indirect effects of introducing these types of non-native organisms. In 1915, Huang (1987) reports that the US Department of Agriculture noted in the south of China that farmers were raising *Oecophylla smaragdima*, ants, to control phytophagous insect pests on orange trees. Farmers would rear these ants, tie strings between trees, release the ants on one tree and they would travel between trees controlling pest outbreaks. Most recently, biological control is growing in the pest management market in greenhouse-grown vegetable crops and also proving successful in ornamental greenhouse production. This change in direction, away from synthetic pesticides, but a response from consumer concern and is an acknowledgment of accumulated negative environmental impact due to years of traditional harsh chemical usage (Pilkington et al., 2009).

Natural biological control is defined as a pest population reduction from a naturally occurring predator or pathogen in a native environment. This relationship is not actively facilitated by human interaction but conceived through evolution and natural changes to the environment over time. There are three strategical approaches, outside of natural biological

control, used for controlling pest populations in diverse production systems. The most common strategy utilized in the greenhouse industry is referred to as augmentative biological control. Augmentative biological control is the periodic release of a biological agent to prevent or actively control a pest population in a given area (Van Lenteren, 2012). Van Lenteren (2012) continues to explain the acceptance of augmentative biological control by referencing a massive amount of pest species, 170 worldwide, that were controlled by periodic releases in 2010 which totaled an application treatment area of 0.4% of all land under cultivation. In the field environment inoculative (classical) and conservation biological control strategies are more successful (van Lenteren, 2012). Classical biological control is when an exotic, non-native, biological control agent is introduced to an environment for long-term establishment and pest control (Eilenberg et al., 2001). Eilenberg et al. (2001) also explains conservation biological control is the manipulation of the physical components of the environment to enhance the survival and persistence of a native biological control agent to reduce pest populations. These specific strategies are chosen to favor the growing conditions, whether in the field or in the greenhouse.

The production of greenhouse cultivated crops has tremendous advantage over traditional seasonally grown field crops. The ability of greenhouse operators to manipulate the greenhouse environment is key to being a successful producer in this market. With specific emphasis on ornamental producers, the advantages of their climate-controlled environment come with its own challenges. Growers have the ability, in greenhouses, to manipulate the growing environment for specific crop requirements during production but also provide the opportunity to facilitate a suitable environment for the implementation of biological controls (Pilkington et al., 2009). As conducive as it is for the successful production of year-around product, the climate-

controlled nature of greenhouses also lends itself to heavy pressure from pests and diseases, even more than field-grown crops in many ways. The high host density in the greenhouse and the fixed technology used for moisture management favor specific types of pests to acclimate, disperse and infest these systems (Pilkington et al., 2009). Throughout the growing year and in between crop rotations, greenhouse operators sterilize the entire greenhouse environment, preventing and killing infestations. Even with these precautions there are still pests that are well suited for this style of growing.

Entomopathogenic Nematodes

Entomopathogenic nematodes (EPNs) in the genera *Steinernema* and *Heterorhabditis* are used commercially as biological control alternatives to many environmentally harsh insecticides (Grewal et al., 2005). These two genera consist of 93 species combined (75 steinernematids and 18 heterorhabditids). Nematodes are roundworms of the phylum Nematoda and some can be described as pest parasitic (entomopathogenic) (Morales-Romas et al., 2014) (van Lenteren, 2012). In the past, the definition of EPNs has referred to only the families Heterorhabditidae and Steinernematidae but it has recently grown to encompass specific species from the genus *Oscheius* (Morales-Romas et al., 2014). These entomopathogens have characteristics in common with insect parasitoids, predators, and microbial pathogens. These characteristics consist of: having a broad host range, being highly virulent and killing quickly, being easily cultured in vitro, having a high reproductive potential, safe to non-target organisms, viable after application through traditional irrigation and spray equipment, being compatible with many chemical pesticides (Kaya, 1993). This thesis will focus on the genera *Steinernema* and *Heterorhabditis* because they are the only ones sold for commercial biological control and the only

entomopathogenic nematodes for which mass production protocols have been developed (Morales-Romas et al., 2014).

Entomopathogenic Bacterial Associations

Each EPN has a "Dauer" juvenile stage which is also referred to as the infectious juvenile (IJ) stage. Entomopathogenic nematodes, are only free-living in this juvenile infectious stage. This juvenile stage is a response to a depleting food source combined with internal host environmental changes (Ehlers, 2001). Each IJ harbors thousands of bacterial symbiont cells in their intestines (Addis et al., 2016). *Steinernema* spp. share a symbiosis with the bacteria genus *Xenorhabdus,* whereas *Heterorhabditis* spp. form a symbiotic relationship with *Photorhabdus*. Once inside the insect, the infective juveniles shield the bacteria from the insect's immune defenses, eventually releasing the bacteria into the circulatory system (haemocoel) of the insect by secretions from their anus. During this process, the nematodes release other toxins that inhibit the immune system of the insect. Ehlers (2001) explained this stage as the recovery stage. After the bacteria have overcome its defense mechanisms, the host insect dies, two days after infection. After the host has died, the environment inside the host becomes suitable for nematode reproduction. The nematodes will feed on the symbiotic cells and develop into adults and produce offspring. After consumption of the symbiont, the offspring now contain this bacteria in the intestine and exit the insect in search of new hosts.

Steinernema feltiae

Steinernema feltiae Filipjev (1934) is a species that consists of multiple strains. These strains originate from Spain (Campos-Herrera et al., 2006) to Indonesia (Griffin et al., 2000) with mass rearing facilities from Germany to Canada (Georgis et al., 2005). The strain used in this thesis was originally isolated in France and was supplied by the USDA-ARS, Dr. David I

Shapiro-Ilan, GA USA. *Steinernema* spp. reproduce by male and female cross fertilization and require symbiotic bacteria to survive, feed and trigger reproduction (Griffin et al., 2000). *Steinernema feltiae* can be mobile throughout the soil profile with mobility defined as infection of different larvae located at different depths of substrate when used for biological control (Susurluk, 2008). Susurluk (2008) found *Steinernema* spp. were mobile at temperatures from 15°C to 25°C. Furthermore, *S. feltiae* proved to be more mobile at 15°C throughout the entire soil profile than at other temperatures. *Steinernema feltiae* is used to treat for fungus gnats (*Bradysia difformis* Frey, 1948) and Western flower thrips (*Frankliniella occidentalis* Pergande, 1895) in greenhouse applications in soilless media.

Steinernema carpocapsae

Steinernema carpocapsae Weiser, 1955, (strain All) is the most common strain of *S. carpocapsae* used for biological control. For this thesis, this strain was isolated in Georgia, USA, and provided by the USDA-ARS. *Steinernema carpocapsae* is used to control shore flies (*Scatella stagnalis* Fallen, 1813) in greenhouse applications. The use of EPN can be a struggle for greenhouse operators because of the limited temperature range in which each genus provides effective control. Siegel et al. (2006) found that the use of *S. carpocapsae* applied through an application volume of 1,870L water/ha was effective when followed by a post irrigation event of the same volume. However, regarding temperature, this application was only successful when temperatures did not exceed 32°C within 24 hours after the application. *Steinernema carpocapsae* is well known for surviving in low moisture environments accompanied by high relative humidity when compared to other *Steinernema* spp. (Zhioua, 1995). *Steinernema carpocapsae*, similar to *S. feltiae* is considered an ambush cursing forager and is effective at finding sedentary resources and hosts (Wilson et al., 2012). *Steinernema carpocapsae* is also

known to stand on its tail in search of olfactory cues which help in the determination of the direction of a host (Bal et al., 2014). Outside of the greenhouse facility, *S. carpocapsae* is well known in the tree industry for control of the codling moth with applications through a hand-gun sprayer (Unruh, 2001). Unruh (2001) describes the process in detail with first concluding that pre-wetting applications with water on infected trees is required followed by gun application of 2 million IJs/tree produce up to 78% mortality of codling moth larvae. *Steinernema* spp. are diverse, easily applied and environmentally friendly biological control agent.

Heterorhabditis bacteriophora

Heterorhabditis bacteriophora have been surveyed across the world and have such a significant distribution that Antarctica is the only site they have not been found (Dolinski et al., 2008). With this type of distribution, there are many strains of this species that can be used for biological control but for this thesis we used HP88 strain supplied by the USDA-ARS which was isolated in the United States in the state of Utah. It is known that the morphological identification of *Heterorhabditis* spp. between species and strains is very difficult (Dolinski et al., 2008). Dolinski et al. (2008) explain that the body and tail lengths of IJs are used to determine difference in species, as well as spicule and gubernaculum length and shape, can further aid in identification. Infective juveniles of *Heterorhabditis* spp. burrow through the inter-segmental membrane of pest larvae with their terminal tooth cutting through the cuticle, which is another morphological characteristic described by Owuama (2001). *Heterorhabditis bacteriophora* demonstrated the same mobility behavior as *S. feltiae,* but at higher temperatures, from 15°C to 25°C (Susurluk, 2008). *Heterorhabditis bacteriophora* are used for biological control of fungus gnats and Western flower thrips (Grewal et al., 2005). Choo (2012) suggests, that in some cases, *H. bacteriophora* is more effective when used in combination with other insect pathogens. When

treating fungus gnats *H. bacteriophora* and *Beauveria bassiana*, used together, resulted in significantly higher insect mortality then either of the insect pathogens alone (Choo, 2012). *Heterorhabditis bacteriophora* possess both automictic hermaphrodites and amphimictic females and males which differ from the genera *Steinernema* and understanding these characteristics aids in the appropriate application type and frequency (Ciche and Sternberg, 2007).

Heterorhabditis indica

Heterorhabditis indica, like many other entomopathogens, is an obligate parasite that maintains many parasitic adaptations (Noguez et al., 2012). One of these adaptations it shares with its cousin, *H. bacteriophora*, a buccal tooth it uses to slice into its host (Noguez et al., 2012). This adaptation allows for more efficient penetration and infection of the host. The understanding of the parasitic adaptations of this genera have included them, not only inside of the greenhouse as biological control agents but also in other areas of production, such as post-harvest (Mbata and Shapiro-Ilan, 2010). Mauleon et al. (2006) explain that ideal nematode survival temperature is 28°C to 29°C, but also described the extreme temperatures of the native habitat of *H. indica* near limestone cliffs in Guadeloupe reach as high as 50°C. This suggests that certain EPN species can tolerate extreme temperatures. EPNs range in virulence and *Heterorhabditis* spp. are reported to have higher virulence than steinernematids. Mbata and Shapiro-Ilan (2010) report that *H. indica* is by far one of the most virulent species that can be used for biological control. Their study reported higher mortality on larvae and adults of *P. interpunctella* when compared against multiple steinernematids.

Crop Quality

The greenhouse industry runs on plant quality. This quality is determined by multiple parties when selecting certain species to be grown but, ultimately, quality is determined by the end consumer when the plants arrive at the retail store. This means the plants are judged after they have gone through the shipping process. Loss of plants during the process of shipping is one of the most significant risks for growers. Shipping conditions can be unpredictable, and damage may vary from physical damage during transit or during loading and unloading to product damaged or destroyed by fungi and other disease organisms. Plants are often under stress due to their placement on closely stacked racks on metal carts in cooled, dark and humid trailers. One of the most problematic fungi affecting quality and causing financial loss and frustration during shipping is *Botrytis cinerea* (Nell, 2016).

Infection by *Botrytis* spp. damages on multiple species of commercially grown potted crops and cut flowers as well as agricultural fruit and vegetable crops. *Botrytis cinerea* can be damaging during propagation and can cause disease on roots and stem bases under conditions of high humidity with the presence of water films, or throughout the growing cycle on flower petals (Elad et al., 2007). Most often, in greenhouse environments, *B. cinerea* causes damage on upper plant parts, including leaves, flowers, fruits, buds and stems. *B. cinerea* can be very active in a broad range of temperatures between 2°C and 30°C, but can survive at temperatures as low as 0°C; thus making *B. cinerea*, which is a vigorous sporulator that produces dry conidia, the most problematic pathogen during postharvest conditions (Elad et al., 2007). Conidia spores can be widely dispersed on air currents. Because of this activity growers are forced to find innovative ways to prevent infection by this pathogen to avoid compromising plant quality.

Growers are trying new strategies to prevent and control the spread of this pathogen. Some are manipulating shipping conditions to reduce relative humidity by changing irrigation strategies so plants do not sit in the trailer with moisture on plant tissue. Some growers are making multiple applications of preventative fungicides during the growing cycle to help the plant become resistant to *Botrytis* infection. Another problem with *Botrytis spp.,* as growers have found out, is that this fungus has been very resistant to certain active ingredients in many different fungicides (Fernandez-Ortuno et al., 2014). Production of ethylene can be a major factor in driving infection through flower senescence (Tanase et al., 2009). Once plants come in contact with and produce high levels of ethylene, their flowers and leaves will start to senesce and drop; which makes plants more susceptible to fungal infection. Add this susceptibility to shipping conditions, cool temperatures, moist plant substrate, and tissue and low to no light and growers create the conducive environment for damage.

Growers have become more efficient at using their cultural practices as tools for control and suppression of many different problematic pathogens. An example of this is changing their irrigation strategies to reduce waterlogging on roots to prevent multiple species of root rot (personal observations, Battlefield Farms). Growers clean all surfaces after using pots, trays, and floors to keep all tools and surfaces clean of spores. Growers are becoming more accountable of their use of pesticides to address consumer concerns. With increasing consumer interest in environmental sustainability, growers are now being pushed to use more "biologically friendly" approaches. One approach that is effective on cut flowers, grapes and lettuce is the use of ultraviolet radiation for prevention and control of *Botrytis spp.* Ultraviolet radiation can kill fungus conidia on contact and also help the crop defend itself against spores penetrating tissue surfaces by stimulating the plant's own defense system (Darras et al., 2009; Nigro et al., 1998;

Ouhibi et al., 2014). UV may aid in promoting disease resistance through the plant's active defense system through a process defined as hormesis, "harmful biological practices may induce beneficial responses when applied at lower doses" (Ouhibi et al., 2014).

Crops

Many ornamental annual crops are sold in the United States that all can have problems with fungal diseases throughout the growing cycle as well as during shipping logistics. The industry's sales in 2015 for floricultural crops totaled $4.37 billion (U.S.D.A, 2016). Of these sales, the top three selling annuals were Geraniums with $24.5 million in sales, Petunias with $56.5 million in sales as well as Begonias with $31.8 million in sales (U.S.D.A., 2016). Based on grower feedback, trade magazines, and large big-box retailers from personal experience in the floricultural/horticultural industries, petunias experience the most damage during transit from fungal disease and geranium is right behind with similar problems during transit. These crops, when damage is noticed at the end retail location, are then worthless to the consumer, and credits are written. These credits are issued to the grower and no sale is made from the crops that show fungal damage.

Petunia: *P. x hybrid*

Petunia spp. are the ornamental crops sold in the highest volume throughout the United States (U.S.D.A., 2015). Petunia can be propagated from seed or vegetative cutting. When propagated from seed, petunia are sown in 288-cell trays with peat-based substrate and placed in a propagation greenhouse (PanAm, 2016). These propagation greenhouses usually have moderate temperatures between 72-76 °F for best germination rates as well as constant mist to keep seeds from drying out and to help with imbibition; which is the rapid increase in water

uptake during germination (Hartmann et al., 2011). Petunia take roughly four to seven days to germinate depending on the cultivar and grow in the plug tray for four to six weeks, also cultivar dependent. There is a very wide range of petunia cultivars with differing habits which dictates total crop time and final pot size. Typically the highest volume used pot size is a four-inch diameter pot for petunias, which are referred to, in the trade, as packs. Petunia x hybrid is transplanted into a four-inch pot and grown for six to seven more weeks until considered finished, in full flower and ready to ship. During the finished growing stage greenhouse temperatures are recommended to stay around 62-65 °F during the day and 55-65 °F during the night. In addition, right after transplant into the finished pot. Growth is controlled through environmental conditions with light, temperature, and moisture control. Some PGRs (plant growth regulators) are used when specific petunia habits need more branching or other morphological changes which environment can't change (Nau, 2011).

Petunias have very large, thin flowers. When growing seed varieties, these flowers are also self-pollinated when compared to vegetative, which are not (Nell, 2016). These characteristics aid in this crop's susceptibility to *Botrytis cinerea* infection. When flowers are pollinated, in this case due to irrigation, handling or even fans which shake petals, flowers start to senesce. These flowers which are senescing are now non-active and use metabolism for seed production. Petals start to die, dry out and fall. These petals are very susceptible to infection from conidia spores. Also, petunias continually produce flowers and these flowers cover the plant. When there are multiple generations of flower much of the dead and dying petals turns into the perfect host for infection.

Begonias: *B. x hybrid*

Begonia spp. are the second-highest selling ornamental annual in the United States (U.S.D.A., 2015). Most begonias are grown from seed. Begonias are sown in a 288-cell tray with peat-based substrate and placed in a propagation greenhouse (PanAm, 2016). These propagation greenhouses usually have moderate temperatures between 72-78 °F for best germination rates. On average, begonias take 7-10 days to germinate in these conditions and grow in the plug stage for 7-8 weeks. When transplanting begonias there are many different pot sizes ranging from four-inch to one-gallon pots with up to three plants per pot and even 10-12 inch pot with up to four plants per pot. During the finished growing stage, greenhouse temperatures are recommended to stay around 65-75 °F during the day and 62-67 °F during the night. Growers very rarely use PGRs on begonias due to their being very sensitive to moisture. Growers can control growth by keeping substrate dry. This is a common practice.

Begonias have many different size flowers depending on the cultivar but most of the time this annual's flowers are very thick and sometimes very compact with many petals growing together. This type of inflorescence can become very susceptible to Botrytis infection when flowers get scorched by the sun or start to die from age or other reasons. When these flowers die, all this tissue becomes a very susceptible tissue environmental for the fungus. Begonias, like petunias, are also prone to get infected with botrytis during shipping with the lack of light, cool temperatures and high moisture.

Geraniums: *Pelargonium x hortorum*

Pelargonium spp., referred to as geraniums are also one of the top three sold annuals in the United States (U.S.D.A., 2015). Geraniums can be propagated by seed, cutting or callus cutting. A successful propagation method with this crop is using callus cuttings. Growers direct stick this type of cutting into the finish pot saving space and labor in the greenhouse. To propagate in the

finish pot, wet pot to a moisture level of four the day before sticking (Syngenta, 2014). Once cuttings arrive direct stick cuttings in the middle of the finish, already moist pots; mist enough to keep plant tissue wet until rooted. Keep temperatures between 70 to 72°F. It is recommended to apply a surfactant to irrigation water to help break the water tension and achieve proper coverage on plant tissue during irrigation and misting. Geraniums are also very susceptible to root diseases as well as rust and bacteria leaf spot which can be prevented with proper preventative applications of fungicides and bactericides and good moisture management. As plants begin to root and grow, lessen misting and start increasing nitrogen for proper fertilization. Temperatures can be reduced at night to 68 to 70°F to energy-saving if needed but this might slow growth and flower development, extending the time of this crop in the greenhouse. As plants start to flower, deadhead until crop is consistently flowering and all flowers in bloom are near the same growth development stage.

Geraniums are problematic when shedding their flowers. This makes for an unsightly plant as well as a conducive host for *Botrytis.* During shipping, these flowers are similar to begonias, above, with many petals and dense flowers. With the dense inflorescence of geraniums, moisture is captured and increased inside this type of flower which allows Botrytis conidia to sit and germinate, destroying flower petal tissue and leading to poor quality flowers when delivered at retail (Syngenta, 2014).

Botrytis (*B. cinerea)*

All *Botrytis spp.,* but specifically *Botrytis cinerea,* are ubiquitous fungi with a necrotrophic lifestyle i.e., pathogen attacks its host and feeds on dead tissue (Elad et al., 2007; Williamson et al., 2007). *B. cinerea* is damaging on over 200 dicotyledonous species, ranging from ornamentals and cut flowers to agricultural crops (Williamson et al., 2007). A common

term used throughout these industries identifies *B. cinerea* infections as visually being grey mold. Even though this fungus is widely known to be devastating in the field, it is also devastating in the controlled environment of commercial greenhouses (Williamson et al., 2007).

B. cinerea can survive and overwinter in temperatures as low as 0°C but is most active to infect plant cells between 2°C and 30°C (Elad et al., 2007). However, *B. cinerea* most adequately progresses through the different growth stages, producing conidia (spores) which actively germinate and infect more cell tissue, in temperatures between 12°C and 30°C. Overwintered sclerotia germinate and mycelium grow to produce conidia which then infect host tissue by windblown or rain-splash transportation. With this temperature range and the ubiquitous nature of this fungus, control does not only hinge on proper temperature management but also humidity, and more specifically, vapor pressure deficit (VPD). Once this fungus germinates, the conidia develop germ tubes that penetrate the tissue, usually penetrating a single cell first, and produce wall-degrading enzymes and toxins which trigger the host into inducing cell death through the host's programmed defenses (Williamson et al., 2007). The conidia spores of *B. cinerea* can travel into the greenhouse through fans, air currents, worker's clothes as well as be dispersed from already infected plant tissue when temperatures change.

One of the major control measures taken to minimize Botrytis infections is keeping floors and tables clean of old plant debris. This is the perfect place for botrytis to develop sclerotia, the overwintering structure of this fungus. Some fungicides are not adequate to control this growth stage and other control strategies must be considered (Williamson et al., 2007). After *B. cinerea* has developed into sclerotia the next growth stage is usually induced with a combination of light, near UV (nUV) radiation, moisture, and temperature changes (Williamson et al., 2007). Some research has found that *B. cinerea* can continue through the growth stages without the presence

of light and nUV, so these factors do not permanently hinder growth (Williamson et al., 2007). From here the fungus progresses, with the appropriate conducive environment triggering its growth, to develop conidiophores and multinucleate conidia. This is now the most active inoculum that will be dispersed and germinate to increase *B. cinerea* epidemics throughout a cropping system. Another form of inoculum is mycelium which can dwell, latently, in hosts and reside in seeds (Williamson et al., 2007).

The different growth stages of *B. cinerea* are expressed differently in different crops. In many crops *B. cinerea* can be harmful at different phases in plant development. Most of the time new plant growth is not nearly as susceptible as older plant tissue (Elad et al., 2007). Mechanical injury and physical harvesting or pinching of crops expose the plant to a higher probability of infection at the wound site. *B. cinerea* is even known to penetrate and travel through the petiole of tomato plants (Elad et al., 2007). The most problematic infection site for many ornamental growers is on the flower petal tissue. *B. cinerea* can infect fragile petal tissue during the dark, cool and moist conditions of shipping which equates to visible imperfections on flowers called lesions. One report conducted on *Freesia hybrida* L. cut flowers called this type of visual infection "specking" (Darras et al., 2010). This is when the plant can contain the infection through provoked defenses, such as phytoalexins, which is called a hypersensitive response. This hypersensitive response confines *Botrytis* spp. to that one area and therefore depletes its nutrient sources and the fungus dies. Many crops are being bred to have stronger and more resistant hypersensitive responses for the sake of ensuring quality. Dummen Orange is trying to breed this hypersensitive response into the new generations of poinsettia cultivars (Ruth Kobayashi, personal communication). Many efforts are being taken to understand, prevent and control this fungus but all live-goods industries still do not have a common environmentally friendly answer.

UV Radiation

Ultraviolet (UV) radiation borders the visual spectrum at the electromagnetic range between 200 to 400 nm. There are three ranges of UV starting closest to the visual spectrum with, UV-A (320-400 nm), into UV-B (280-320 nm), then lastly into UV-C (200-280 nm) (Mc Donald, 2003). Ninety-three percent of the radiation that reaches the earth's surface is between 400-750 nm with the other 7% being the UV-A and UV-B spectrum between 295 nm to 400 nm. UV-C does not reach the earth's surface (McDonald, 2003). Plant growth on this planet is partially due to the filtration of UV through the ozone layer. The ozone layer filters out the entire UV-C and part of the UV-B spectrums. UV alters or hinders plant photosynthetic function through damaging the chloroplasts. All radiation is biologically active with UV included; thus the damage results from the dose, duration, and density of a specific quality of radiation received by the plant at a given time (Ouhibi et al., 2014). UV absorbed at high doses results in damaging effects whereas when applied and absorbed at a hormic dose can provide some biologically beneficial responses. Some produce farmers use UV-C at hormic doses, a short, intense dose, for extending shelf life of harvested fruits and vegetables (Ouhibi et al., 2014). There are multiple reasons for this type of application, ranging from cell death to make the plant tissue harder to be penetrated by fungi to plants being induced to produce defense compounds making it more disease tolerant which prolongs its shelf life (Ouhibi et al., 2014). UV-C applied to a strawberry retards softening or enhances quality when applied to a mango (Ouhibi et al., 2014). UV-C was used at specific doses to induce disease resistance to *Botrytis cinerea* during shipping on table grapes (Nigro et al., 1998).

Not only plants are affected by UV radiation but UV also causes ultrastructure cellular effects in most organisms. *Botrytis cinerea*, which causes grey mold on many harvested crops,

can be quite sensitive to radiation in the UV spectrum (Darras et al., 2009). Direct exposure to UV-C, also referred to as germicidal wavelengths, can irradiate pathogens in combination with induction of host defenses (Darras et al., 2009). UV-C can cause *B. cinerea* conidia to inactivate which will lessen the severity of overall infection. A study conducted on cut flower freesia concluded that UV-C irradiation after artificial inoculation of *B. cinerea* on inflorescences resulted in lower disease severity than when UV-C was applied before artificial inoculation (Darras et al., 2009).

There have been many studies with UV-C for disease management that include many different species of fruit crops. For example, a study on watermelon showed that when UV-C is applied at low doses (1.6 and 2.8 kJ/m), the fruit can be stored for up to 11 days at 5°C before significant fungal infection (Ouhibi et al., 2014). This strategy of pre-application of UV-C has been used on many crops to prevent fungal decay. Some of the crops include carrots, citrus, and tomatoes where UV-C induces disease resistance through the accumulation of phytoalexins (Ouhibi et al., 2014). In a study using Romaine lettuce, *Lactuca sativa* L., applying UV-C (0.85 kJ/m^2) after harvest and before shipping significantly reduced the size of *Botrytis cinerea* infected lesions on leaves continually for up to four days as compared to the control which received no UV-C exposure (Ouhibi et al., 2014). In addition to fungal pathogens, bacterial pathogens such as soft rot (*Pseudomonas spp.*) can cause significant crop damage and losses (Escalona et al., 2010). UV-C has been tested on this type of pathogen as well with a study using baby spinach, wherein almost all of the signs of the pathogens were reduced with exposure to UV-C at the beginning of storage (Escalona et al., 2010). Another significant example of UV-C's ability is in its use on grapes during shipping. As stated previously most all shipping rots can cause high economic losses (Nigro et al., 1998). A study conducted in Italy found that applying

UV-C to table grapes (*Vitis vinifera* L. cv. Italia) resulted in lower total infection and smaller lesion diameter (with time of inoculation being a major factor) (Nigro et al., 1998). Even though it is known that UV can be very dangerous at high doses, altering DNA, causing mutations as well as disturbing biological processes, UV can also be harnessed to prevent disease damage and ensure quality during postharvest conditions (Ouhibi et al., 2014)

Literature Cited

Addis, T., A. Teshome, O. Strauch and R. Ehlers. 2016. Life history trait analysis of the entomopathogenic nematode *Steinernema feltiae* provides the basis for prediction of dauer juveniles yields in monoxenic liquid culture. Appl Microbiol Biotechnol 100(20016):4357-4366.

Bai, W., B. J. Adams, T. A. Ciche, S. Clifton, R. Gaugler, K. Kim, J. Spieth, P. W. Sternberg, R. K. Wilson and P. S. Grewal. 2013. A lover and fighter: The genome sequence of an entomopathogenic nematode *Heterorhabditis bacteriophora*. PLoS One 8(7): 1-13.

Campos-Herrera, R., M. Escuer, L. Robertson and C. Gutierrez. 2006. Morphological and ecological characterization of *Steinernema feltiae* (Rhabditida: Steinernematidae) Rioja strain isolated from *Bibio hortulanus* (Diptera: Bibionidae) in Spain. Journal of Nematology 38(1): 68-75

Choo, H. Y., H. K. Kaya, J. Huh, D. W. Lee, H.H. Kim, S. M. Lee and Y. M. Choo. 2002. Entomopathogenic nematodes (*Steinernema* spp. and *Heterorhabditis bacteriophora*) and a fungus *Beauveria brongniatartii* for biological control of the white grubs, *Ectinohoplia rufipes* and *Exomala orientalis*, in Korean golf courses.

Ciche, A. T., and P. W. Sternberg. 2007. Postembryonic PNAi in Heterorhabditis bacteriophora: a nematode insect parasite and host for insect pathogenic symbionts. BMC Development Biology 7(2007):101

Cruz-Martinez, H., J. Ruiz-Vega, P.T. Matadamas-Ortiz, C. I. Cortes-Martinez and J. Rosas-Diaz. 2017. Formulation of entomopathogenic nematodes for crop pest control- a review. Plant Protect. Sci. 53 (2017):15-24.

Darras, A.I., D.C. Joyce and L.A. Terry. 2010. Postharvest UV-C irradiation on cut *Freesia hybrid* L. inflorescences suppress petal specking caused by *Botrytis cinerea.* Postharvest Biol. Technol. 55(2010):186-188.

Dolinski, C., F. L. Kamitani, I. R. Machado and C. E. Winter. 2008. Molecular and morphological characterization of heterorhabditid entomopathogenic nematodes from the tropical rainforest in Brazil. Mem Inst Oswaldo Cruz, Rio de Janerio. 103(2): 150-159.

Ehlers, R.U. 2001. Mass production of entomopathogenic nematodes for plant protection. Appl Microbiol Biotechnol 56 (2001): 623-633.

Eilenberg, J., A. Hajek and C. Lomer. 2001. Suggestions for unifying the terminology in biological control. BioControl 46 (2001):387-400.

Elad, Y. 1988. Latent infection of *Botrytis cinerea* in rose flowers and combined chemical and physiological control of the disease. Crop Protection 7(6):361-366.

Elad, Y., B. Williamson, P. Tudzynski and N. Delen. 2007. *Botrytis* spp. and diseases they cause in agricultural systems – An introduction, p. 1-8. In: Elad, Y., B. Williamson, P. Tudzynski and

N. Delen (eds.). *Botrytis:* Biology, Pathology and Control. Springer, Dordrecht, The Netherlands.

Elad, Y., B. Williamson, P. Tudzynski and N. Delen. 2007. Epidemiology of *Botrytis cinerea* diseases in greenhouses, p. 319-333. Botrytis: Biology Pathology and Control. Springer, Dordrecht, The Netherlands.

Escalona, V.H., E. Aguayo, G.B. Martinez-Hernandez and F. Artes. 2010. UV-C doses to reduce pathogen spoilage bacterial growth *in vitro* and in baby spinach. Postharvest Biology and Technology 56(2010):223-231

Fernandez-Ortuno, D., Grabke, A., Bryson, P.K., Amiri, A., Peres, N. A., Schnabel, G. 2014. Fungicide resistance profile in *Botrytis cinerea* from strawberry fields of seven southern U.S. States. Plant Disease 98: 825-833.

Gal, H. K., and A. P. Michel and P. S. Grewal. 2014. Genetic selection of the ambush foraging entomopathogenic nematode, *Steinernema carpocapsae* for enhanced dispersal and its associated trade-offs. Evol Ecol 28(2014): 923-939.

Georgis, R., A. M. Koppenhofer, L. A. Lacey, G. Belair, L. W. Duncan, P. S. Grewal, M. Samish, L. Tan, P. Torr, and R. W. H. M. van Tol. 2006. Successes and failures in the use of parasitic nematodes for pest control. Biol. Control 38(2006): 103-123.

Griffin, C.T., K. M. O'Callaghan and I. Dix. 2000. A self-fertile species of *Steinernema* from Indonesia: further evidence of convergent evolution amongst entomopathogenic nematode? Parasitology 122(2001): 181-186.

Hajek, A. E., B. P. Hurley, M. Kenis, J. R. Garnas, S. J. Bush, M. J. Wingfield, J. C. van Lenteren and M. J. W. Cock. 2016. Exotic biological control agents: A solution or contribution to arthropod invasions? 18 (2016): 953-969.

Hartman, H. T., D. E. Kester, F. T. Davis Jr., R. L. Geneve. 2011. Principles of Propagation from Seeds, p. 201. Hartmann & Kester's Plant Propagation Principles and Practices. Pearson, Upper Saddle River, NJ.

Huang, H. T. and P. Yang. 1987. The ancient cultured citrus ant. American institute of biological sciences. 37 (9) : 665-671.

Kaya, H. K. 1993. Entomopathogenic Nematodes. Ann. Rev. Entomol. 38(1993):181-206.

Marquenie, D., J. Lammertyn, A.H. Geeraerd, C. Soontjens, J.F. Van Impe, B.M. Nicolai and C.W. Michiels. 2002. Inactivation of conidia of *Botrytis cinerea* and *Monilinia fructigena* using UV-C and heat treatment. International Journal of Food Microbiology 74(2002):27-35.

Mbata, G. N., and D. I. Shapiro-Ilan. 2010. Compatibility of *Heterorhabditis indica* (Rhabditida: Heterorhabditidae) and *Habrobracon hebetor* (Hymenoptera: Braconidae) for biological control of *Plodia interpunctella* (Lepidoptera: Pyralidae). Biol. Control 54(2012):75-82.

Mc Donald, M.S. 2003. Photomorphogenesis, p. 221-223. Photobiology of Higher Plants. John Wiley & Sons Inc., Hoboken, NJ.

Morales-Romas, J. A., M. G. Rojas and D. I. Shapiro-Ilan. 2014. Production of entomopathogenic nematodes, p.321-355. In: D. I. Shapiro-Ilan, R. Han and X. Qiu. Mass production of beneficial organisms: invertebrates and entomopathogens. Academic Press, London, UK.

Nau, J., 2011. Propagating seed crops, p. 143-154. Ball Red Book, Volume 2. Ball Publishing, West Chicago, IL.

Nell, A. T. 2016. Preventing petunia flower melt. Grower Talks, p. 48-54. Vol. 79, No. 10.

Nigro, F., A. Ippolito, G. Lima. 1998. Use of UV-C light to reduce *Botrytis* storage rot of table grapes. Postharvest Biology and Technology 13(1998):171-181.

Noguez, J. H., E. S. Conner, Y. Zhou, T. A. Ciche, J. R. Ragains and Rebecca A. Butcher. 2012. A novel ascaroside controls the parasitic life cycle of the entomopathogenic nematode *Heterorhabditis bacteriophora*. ACS Chemical Biology 7(2012):961-966.

Ouhibi, C., H. Attia, P. Nicot, L. Urban, M. Lachaal and J. Aarrouf. 2014. Effect of UV-C radiation on resistance of romaine lettuce (*Lactuca sativa* L.) against *Botrytis cinerea* and *Sclerotinia minor*. J. Phytopathol. 163(2015):578-582.

PanAmerican Seed. 2016. Annual culture chart, p. 36-37. 2016 Seed Production information Guide. Ball Horticulture Company, West Chicago, IL.

Pilkington, L. J., G. Messelink, J.C. van Lenteren and K. Le Mottee. 2009. Protected biological control – Biological pest management in the greenhouse industry. Biological Control 52 (2010):216-220.

Raviv, M. and Y. Antignus. 2004. UV radiation on pathogens and insect pests of greenhouse-grown crops. Photochem. Photobiol. 79(3):219-226.

Suthaparan, A., A. Stensvand, K. A. Solhaug, L.M. Mortensen, R.C. Seem, H. R. Gislerod, S. Torre, D.M. Gadoury. 2012. Suppression of Powdery Mildew (*Podosphaera pannosa*) in Greenhouse Roses by Brief Exposure to Supplemental UV-B radiation. The American Phytopathological Society. Plant Disease (November 2012).

Syngenta, 2014. Cutting time production: Tango geranium. GoldfischVegetative. Syngenta Flowers.

Tanase, K., K. Tokuhiro, M. Amano, K. Ichimura. 2009. Ethylene sensitivity and changes in ethylene production during senescence in long-lived *Delphinium* flowers without sepal abscission. Postharvest Biol. And Technol. 52(2009):310-312.

Unruh, T. R. and L. A. Lacey. 2001. Control of Codling Moth, (Lepidoptera: Tortricidae), with effects of supplemental wetting and pupation site on infection rate. Biol. Control 20: 48-56.

Van Lenteren, J. C., 2012. The state of commercial augmentative biological control: Plenty of natural enemies, but a frustrating lack of uptake. BioControl 57(2012):1-20.

Williamson, B., B. Tudzynski, P. Tudzynski, and J.A.L. Van Kan. 2007. Botrytis cinerea: the cause of grey mould disease. Molecular Plant Pathology 8(5):561-580.

Wilson, M. J., R. E. and I. Glazer. 2012. Entomopathogenic nematode foraging strategies – is *Steinernema carpocapsae* really an ambush forager. Nematology 14(4):389-394.

Zhioua, E., R. A. Lebrun, H. S. Ginsberg and A. Aeschlimann. 1995. Pathogenicity of *Steinernema carpocapsae* and *S. glaseri* (Nematoda: Steinernematidae) to *Ixodes scapularis* (Acari:Ixodidae). Medical Entomol. 32(1995): 900-905.

Chapter 2: Rearing, harvesting and storage of entomopathogenic nematodes for use in ornamental greenhouse crop protection.

Abstract

A rearing strategy and storage method was tested for high density, multi-species rearing and short term storage (4°C for five days) of infective juveniles of entomopathogenic nematodes (EPN) *Steinernema feltiae*, *Steinernema carpocapsae*, *Heterorhabditis bacteriophora* and *Heterorhabditis indica*. Wax moth larvae (*Galleria mellonella*) were inoculated with each species of EPN. The wax moth larvae infected with EPN were incubated at 22°C until infective juvenile emergence was observed. Once infective juvenile emergence was noticed two samples were taken to count infective juvenile emergence. Then infected wax moth larvae and infective juveniles together were stored for five days at 4°C with two samples being taken daily from host and host substrate during storage to check infective juvenile viability. All four species survived throughout all five days of storage. *Steinernema carpocapsae* produced the least number of infective juveniles in these conditions whereas *S. feltiae* produced the greatest number of infective juveniles across all four species tested. *Heterorhabditis indica* and *H. bacterophora* were most similar in counts produced throughout storage duration. All four species can be successfully reared in large quantities from wax moth larvae and stored for five days at 4°C.

Introduction

Fungus gnats (*Bradysia difformis* Frey, 1948), Western flower thrips (*Frankliniella occidentalis* Pergande, 1895), and shore flies (*Scatella stagnalis* Fallen, 1813*)* are considered three major greenhouse pests in the floricultural industry. The control of these pests under greenhouse conditions has been successful through the use of entomopathogenic nematodes (EPN) (Grewal et al., 2005). *Steinernema* and *Heterorhabditis* are used commercially as biological control alternatives to many environmentally harsh insecticides (Grewal et al., 2005).

These two genera consist of many different species, but this study focuses on the successful production of the following four: *S. feltiae* Filipjev, 1934 (SF), *S. carpocapsae* Weiser, 1955 (SC), *H. bacteriophora* Poinar, 1976 (HB) and *H. indica* Poinar, Karunaker and David, 1992 (HI).

Steinernema feltiae is commonly used for control of fungus gnats and Western flower thrips and *S. carpocapsae* for control of shore fly. *Heterorhabditis bacteriophora* and *H. indica* are used for control of fungus gnats and Western flower thrips (Grewal et al., 2005). *Steinernema* spp. penetrate pest larvae through openings such as the mouth, spiracles, and anus as infectious third-stage juveniles (IJ), the only stage that can survive outside of the host (Owuama, 2001). Infectious juveniles of *Heterorhabditis* spp. burrow through the inter-segmental membrane of pest larvae with their terminal tooth cutting through the cuticle (Owuama, 2001). Unlike other bio-control organisms, such as *Bacillus thuringiensis* Berliner, 1915 and *B. sphaericus* Meyer and Neide, 1904, which invade insect hosts by being consumed, EPNs, once inside the host, inoculate them with entomopathogenic bacteria. In a process detailed by Owuama (2001), *Steinernema* spp. share a symbiotic relationship with the bacterial genus *Xenorhabdus,* whereas *Heterorhabditis* spp. form a symbiotic relationship with *Photorhabdus*. Once inside the insect, the infectious juveniles shield the bacteria from the host pest's immune defenses but eventually release the bacteria into the circulatory system (haemocoel) of the insect. During this process, the nematodes release other toxins that inhibit the immune system of the insect. Ehlers (2001) explained this stage as the recovery stage. After the bacteria have overcome the insect's defense mechanisms, the host dies two days after infection. After the initial infection and death of the host, the environment inside the host becomes suitable for nematode reproduction. When nutrients are available, the nematodes will feed on the symbiotic bacterial cells and mature into

adults, and offspring are produced. After all nutrients are consumed, the offspring retain the symbiotic bacteria in the intestine and leave the insect cadaver in search of new hosts.

The use of EPNs can be a struggle for greenhouse operators because of the limited temperature range in which each genus provides effective control. These two different genera, *Steinernema* and *Heterorhabditis,* are effective and mobile at different temperatures. Siegel et al. (2006) found that the use of *S. carpocapsae* applied through an application volume of 1,870 L water/ha was effective when followed by a post irrigation event of the same volume. However, with regard to temperature, this application was only successful when temperatures did not exceed 32°C within 24 hours after the application. Mauleon et al. (2006) explain that ideal nematode survival temperature is 28°C to 29°C, but also described the extreme temperatures of the native habitat of *H. indica* near limestone cliffs in Guadeloupe which reach as high as 50°C. This suggests that certain EPN species can tolerate extreme temperatures. *Steinernema feltiae* and *H. bacteriophora,* can be mobile throughout the soil profile with mobility defined as infection of different larvae located at different depths (Susurluk, 2008). Susurluk (2008) found both *Steinernema* spp. and *Heterorhabditis* spp. were mobile at temperatures from 15°C to 25°C. Furthermore, *S. feltiae* proved to be more mobile at 15°C throughout the entire soil profile than *H. bacteriophora,* which only traveled within the first few centimeters of soil at this temperature. *Heterorhabditis bacteriophora* demonstrated the same mobility behavior as *S. feltiae,* but at the warmer temperatures, near 25°C (Susurluk, 2008). These studies suggest that when EPNs are exposed to their ideal temperature and applied appropriately, they are highly mobile, and pest control may be increasingly effective when these EPNs are used together at a range of temperatures. This would allow for year-round greenhouse application with multiple hosts targeted, therefore, we included all four species in our research.

Our initial objective was to determine if infectious juveniles of these four EPNs can survive and continue to emerge from the host under cold storage conditions for a normal workweek (5 days). We hypothesize that *S. feltiae* and *S. carpocapsae* will survive and produce steady counts of IJs during all five days of storage due to their recorded cold tolerance and anhydrobiosis (Ali and Wharton, 2012; Andalo et al., 2010). Anhydrobiosis is a physiologically arrested state of dormancy caused by lack of water (Grewal, 2000). We also hypothesize that *H. bacteriophora* and *H. indica* will survive in high numbers, but will decline as they are stored in 4°C because they thrive in warm temperatures (Susurluk, 2008). With these results, our goal is to develop a more efficient rearing and extraction system that is adaptable to greenhouse production practices.

Materials and Methods

Rearing, storage and extraction of ENPs

To determine if infectious juveniles of EPNs can survive and reproduce under cold storage conditions, three rearing laboratory replications were conducted over time. Four EPN species were used, *Steinernema feltiae, Steinernema carpocapsae, Heterorhabditis bacteriophora* and *Heterorhabditis indica.* For each replication of this experiment, there were four separate 25 L sealed plastic containers, one for each species of EPN. On day one, 1,250 wax moth larvae (*Galleria mellonella*) (Knutson's Recreational Sales Inc., Brooklyn, Michigan) in a sawdust substrate, were poured into each of the four separate plastic containers. We received liquid inoculum of each EPN species in plastic bottles from BDS (Biological Defense Systems LLC, Fair Play, South Carolina). We received a separate bottle of inoculum for each species which contained 115 ml of distilled water with approximately 1.7 million infectious juveniles.

One bottle of each species of infectious juveniles was poured into the corresponding plastic container and hand-mixed with sawdust substrate to inoculate the host wax moth larvae.

After inoculations plastic containers were sealed and placed in the lab for 17 days, until infectious juvenile emergence was observed as a thin film on the sides of the containers. During the incubation stage, the lab was kept at a constant temperature of 22°C with ambient light levels. On day 17, containers were taken from the lab to the cooler facility where the containers were stored for five days to mimic a typical work week of Monday to Friday.

Samples were taken by hand mixing the contents in the containers and scooping a 100-ml sample of wax moth larvae, infectious juveniles and sawdust substrate out of each plastic container with a glass beaker. We added 100 ml of room temperature tap water to the sample and let it stand for 30 minutes. After 30 minutes, the contents of the beaker were vigorously mixed and sieved through a 40-mesh screen which extracted the nematodes into a liquid suspension. A 25-µl sample was removed from the suspension and diluted with 10 ml of tap water from which two 25-µl sub-samples were counted for the total number of infectious juveniles using 100x magnification. After the first samples were collected, the plastic containers were placed into a 4°C cooler for five days and two samples were collected and counted each day.

At the conclusion of the incubation stage (Day 17) for each replication, we inoculated the next replicate to begin the incubation stage. The experiment consisted of three replications and count data were compared using Student's t-test using JMP SAS Pro and comparative boxplots or regression analysis (R Core Team, 2013).

Liquid extraction of EPNs

After evaluation of our rearing procedure in a commercial greenhouse, we determined that growers needed a simplified system of extraction. Due to the lack of mortality of fungus gnats and Western flower thrips during commercial trials of the individual EPN species, *H. indica* was excluded from this trial.

Two laboratory replications were conducted during 18 days. For each replication of this experiment there were three 25 L sealed plastic containers, one for each EPN species, *S. feltiae, S. carpocapsae* and *H. bacteriophora.* We received separate liquid inoculum of each species in clear, flat plastic flasks which contained 115 ml of distilled water with approximately 1.7 million infectious juveniles from the USDA (David Shapiro-Ilan, USDA, Byron GA). On day one, one flask of each species of infectious juveniles, *S. feltiae, S. carpocapsae* and *H. bacteriophora,* were poured into three separate 18.9 L plastic buckets and mixed with 1,250 wax moth larvae in a sawdust substrate (Knutson's Recreational Sales Inc.). Each of the three 18.9 L buckets were then hand-mixed with 250 ml of distilled water to thoroughly moisten the substrate and evenly distribute the EPNs. Once substrate and larvae were well moistened with the inoculum, we portioned out the mix into ten 147 ml PET cups per 18.9 L bucket. The ten cups were placed in each of the three 25 L sealed plastic containers, one EPN species per container.

After inoculation, plastic containers were sealed and kept in a lab at 22°C with three holes in the lid filled with cotton to allow air exchange and reduce pest contamination during incubation. Based on preliminary trials, each species started to emerge from the host after different incubation durations with this strategy: on day 9, *S. feltiae* emerged from the host; on day 10, *S. carpocapsae* emerged from the host; and *H. bacteriophora* emerged from the host on day 13. On the day of initial emergence, 946 ml of distilled water was poured in the bottom of the 25 L plastic container. This water was used to rinse the bases of the cups and sides of the

containers to extract all infectious juveniles that emerged from the host without disturbing the host and substrate in the cups (Figure 2-1a, b & c). After rinsing the outside of the cups in the container, the cups were taken out of the container and placed on the container lid so that the sides of the containers could be more vigorously rinsed without overturning the cups. This extraction technique was used every day for 7 days after emergence (DAE) of IJs, for each species. After rinsing, the concentrated solution of IJs was transferred to a 37.8 L bucket containing 34.8 L of water, totaling 35.7 L after inclusion of the liquid EPN suspension. This volume of water left 1.5 L of space empty in the top of the bucket to allow mixing without spilling Mixing was conducted with a large spatula that reached the bottom of the container. From the 35.7 L suspension, three 250 ml aliquots were taken and from this, two separate 25 µl aliquots were taken for a total of six counted samples per species. Infectious juveniles were counted under a 10x lens microscope, daily for 7 days after emergence (DAE). Nematode counts were determined by number of active or curved infectious juveniles. Infectious juveniles that were observed to be laying completely straight, physically of still IJs were considered dead and were not counted. See sample distribution in Figure 2-2.

Statistical Analysis

Linear mixed model with repeated measures ANOVA (with an autoregressive one covariance structure) was used to analyze IJ count data. Error bars were constructed using a 95% confidence interval and comparisons for each pair using Students t-test. Analyses were performed in SAS JMP Pro (SAS Institute, Cary NC) and R (R Core Team 2012).

Results.

Rearing, storage and extraction of EPNs

All four EPN species survived the 6-day storage period. The average daily nematode counts for each species were significantly different (Figure 2-3). *Heterorhabditis bacteriophora* produced 24 more infectious juveniles per day than *H. indica. Steinernema feltiae* had 183 more infectious juveniles per day than did S. *carpocapsae.* When all four species are compared, *S. feltiae* had a greater number of infectious juveniles emerge from the wax moth cadavers and *S. carpocapsae* had the least. Within each species there were no significant replication effects on total number of infectious juveniles or survival of each species through the five days of storage.

The interactions between total counts by day and species are shown in Figure 2-4. *Steinernema carpocapsae*, *H. bacteriophora* and *H. indica* did not have a significant change in infectious juvenile emergence over the 6 days of storage. Based on these results, each of these species can be stored after infectious juvenile emergence is observed, for up to 6 days without a decline in nematode counts. *Steinernema feltiae*, however, had significant differences in emergence between days during storage.

Since there is a noticeable inconsistency in nematode counts within *S. feltiae* (Figure 2-4), each replication of *S. feltiae* was evaluated by day and shown against the other three species (Figure 2-5). These data show a positive trend in samples of nematode counts across each replication and *S. feltiae* shows a positive slope with a R-squared of 0.9472. This trend suggests that even though nematode counts seemed inconsistent with a corresponding day, the total mean number of counted *S. feltiae* nematodes actually increased over the storage period. This trend seems to not be consistent or as obvisous when comparing the other three species shown in the same figure.

Liquid extraction of EPNs

As expected, all three species of EPN produced infective juveniles. However, each species, as seen in Figure 2-6, has significantly different average nematode counts (IJ production) per day for the 7 days after emergence. *Heterorhabditis bacteriophora* produced significantly more IJs than either of the other EPN species. *Steinernema carpocapsae* produced the least out of the three with *S. feltiae* having significantly higher nematode counts than *S. carpocapsae*.

All three EPN species IJs were mobile outside of the host for an extraction period of 7 days after initial emergence, and comparing the three EPN species reveals differing IJ production trends per species (Figures 2-7, 2-8, and 2-9). *Heterorhabditis bacteriophora* had the highest mean nematode count of all species on 2 DAE and significantly decreased on 3, 4 and 5 DAE (Figure 2-7). On 6 and 7 DAE, *H. bacteriophora* nematode counts increased slightly. *Steinernema feltiae* was the only species out of the three to follow a normal distribution with 1 and 7 DAE not being significantly different and counts on 4 DAE significantly higher than all other days (Fig. 2-8). *Steinernema carpocapsae*, on 1 DAE, produced the greatest number of IJs with a steady decline through 7 DAE (Figure. 2-9).

Steinernema feltiae produced the second-highest mean nematode count of the three EPN species as shown in Figure 2-6. During the 7 days after emergence, mean nematode counts of *S. feltiae* fit a normal distribution with 1 DAE and 7 DAE being the same but significantly lower than all other days (Figure 2-8). Nematode counts at 4 DAE were significantly higher than at 1, 6 or 7 DAE and were not significantly different than those at 2, 3 or 5 DAE.

Discussion.

Understanding IJ quantity and appropriate storage conditions for EPN survival is critical to successfully using these microorganisms for biological control of greenhouse pests. From the results of our cold storage trial (Figure 2-3), *S. feltiae* produced more infectious juveniles than the other species reared under these same conditions. *Heterorhabditis spp.* were more similar in infectious juvenile production as a genus than was *Steinernema* in which *S. carpocapsae* produced fewer infectious juveniles under these conditions than *S. feltiae*. These results (Figure 2-4) demonstrate that all four species can be stored in the host and on host substrate at 4°C for up to five days after infectious juvenile emergence without decline in viability. However, the number of infectious juveniles did not increase significantly over storage time, suggesting that there was no continual emergence from the hosts. And, since there was no decline in nematode counts based on our data, perhaps the rearing system would have continued to produce infectious juveniles if they were maintained in the laboratory at 22°C. Hazir et al. (2001) also observed production of *Steinernema* spp. under 22°C but also observed, at 30°C, there was no production nor infectivity of the host. We propose that additional generations may have emerged with additional time under laboratory conditions, totaling much larger numbers of infectious juveniles. In further work on these species, up to three or four additional generations emerged over 3 to 5 additional days at room temperature (personal observation, data not presented).

Also, during this cold storage experiment the contents in the plastic containers were hand mixed each day before the samples were taken. Our intent was to make sure our samples were homogeneously mixed to represent the entire container. However, by doing so we disturbed the wax moth larvae positions on the substrate and may have split some of the cadavers open, thereby interfering with EPN reproduction. Subsequent tests found much higher numbers of

infectious juveniles emerge when the substrate is undisturbed (personal observation, data not presented).

Unlike the other EPN species *S. feltiae* can continue to produce infectious juveniles from the cadavers throughout the five days, even in the cooler under the rigorous physical conditions of hand mixing. Ali and Wharton (2016) studied the freeze tolerance of *S. feltiae,* characterizing its recrystallization inhibition, thermal hysteresis and ice nucleation. They found that *S. feltiae* infectious juveniles were able to survive intracellular ice formation. The temperatures in our experiment were not nearly as extreme as in the study by Ali and Wharton (2016), but this may explain the EPN's ability to, not only survive at low temperatures but also continue to reproduce.

Under the liquid extraction method of harvesting, *H. bacteriophora* can be highly mobile and produce excessive amounts of IJs when undisturbed during a 7-day harvest period at 22°C. Chen et al. (2003) also concluded the same trend in mobility during their study with *Heterorhabditis* spp. being highly mobile in comparison to *Steinernema* spp. Shapiro-Ilan and Gaugler (2002) found a similar trend in IJ production when comparing the two genera reared in liquid culture with *Heterorhabditis bacteriophora* producing 4.3 times more IJs than *Steinernema feltiae*. We observed this trend in our study, not only by the counts (Figure 7), but also by the greater IJ coverage on the containers compared to the other two species. Studies conducted by Biological Defense Systems, LLC., found a similar trend with *Heterorhabditis* spp. and a spike of excessive IJ emergence early in the harvest period when compared directly to *Steinernema* spp. (A. Justice, personal communications). Dr. Justice observed another trend among *Steinernema* spp which supports our findings; a small burst of emergence early in the harvest period, not near as high as *Heterorhabditis* spp., with a steady emergence throughout the

harvest period. In our studies, we found *S. carpocapsae* (Figure 2-9) to follow this same trend of high quantities earlier in the emergence period with counts tapering off during a 7-day period. However, *S. feltiae* (Figure 2-8) proved to differ from *S. carpocapsae* with a normal distribution for IJ emergence.

When comparing these different methods of rearing, harvesting and storage, we find that there can be multiple strategies for extracting and harvesting EPNs from a live host in vitro. Extracting directly from the wax worm hosts disrupts the reproductive process of the EPNs and thus reduces the overall number of IJs that can be extracted during an extraction period. Liquid extraction of EPNs after migration away from the wax worm was an adapted approach from the first study to reduce disruption of the host and take advantage of EPN mobility. This strategy seems to produce more IJs per week than the first study with *H. bacteriophora* IJs traveling far from the cadaver in search of another host.

The ability of ornamental greenhouse operators to manipulate the environment and crop substrate with sustainable precision continues to be a challenge. The incentives to optimize the economic and effective use of these microorganisms will constantly be increasing in years to come. We hope this study helps in the development of protocols for rearing entomopathogenic nematodes in commercial settings to support the success of environmentally sustainable crop production.

Literature Cited

Ali, F. and D.A. Wharton. 2016. Ice-active substances from the infective juveniles of the freeze-tolerant entomopathogenic nematode, *Steinernema feltiae*. PLoS ONE 11(5):e0156502.

Ali, F. and D.A. Wharton. 2012. Cold tolerance of two entomopathogenic nematodes, *Steinernema feltiae* and *Heterorhabditis bacteriophora*. Cryobiology 66(2013):24-29.

Andalo, V., R.S. Cavalcanti, J.P. Molina and A. Moino. 2010. Substrates for storing entomopathogenic nematodes (Rhabditida: Steinernematidae, Heterorhabditidae). Scientific Agricola. 67(3):342-347.

Chen, S. J. LI, X. Han and M. Moens. 2003. Effect of temperature on the pathogenicity of entomopathogenic nematodes (Steinernema and *Heterorhabditis* spp.) to *Delia radicum*. BioCont. 48 (2003):713-724.

Ehlers, R-U. 2001. Mass production of entomopathogenic nematodes for plant protection. Microbiol. Biotechnol. 56(2001):623-633.

Grewal, P.S. 2000. Enhanced ambient storage stability of an entomopathogenic nematode through anhydrobiosis. Pest Mgt. Sci. 56(2000):401-406.

Grewal, P.S., P. Ehlers and D.I. Shapiro-Ilan. 2005. Greenhouse applications, p. 149-166. In: Tomalak, M. (eds.). Nematodes as biocontrol agents. CAB International, Cambridge, MA.

Hazir, S., S. P. Stock, H. K. Kaya, A. M. Koppenhofer and N. Keskin. 2001. Developmental temperature effects on five geographic isolates of the entomopathogenic nematode *Steinernema feltiae* (Nematoda: Steinernematidae) Journal of Invert. Pathol. 77 (2001) 243-250.

Justice, A. 2016. Biological Defense Systems LLC. Personal Communication.

Mauleon, H., D. Denon, and S. Briand. 2006. Spatial and temporal distribution of *Heterorhabditis indica* in their natural habitats of Guadeloupe. Nematology 8(4):603-617.

Owuama, C.I. 2001. Entomopathogenic symbiotic bacteria, *Xenorhabdus* and *Photorhabdus* of nematodes. Microbiol. Biotechnol. 17(2001):505-515.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna Austria. ISBN 3-900051-07-0, URL: http://www.R-project.org/.

Shapiro-Ilan, D. and R Gaugler. 2002. Production technology for entomopathogenic nematodes and their bacterial symbionts. Indust. Microbiol. & Biotchnol. 28 (2002):137-146.

Siegel, J.P., L.A. Lacey, B.S. Higgbee, P. Nobile, and R. Fritts Jr. 2006. Effect of application rates and abiotic factors on *Steinernema carpocapsae* for control of overwintering navel orangeworm (Lepidoptera: Pyralidae, *Amyelois transitella*) in pistachios. Biol. Control 36(2006):324-330.

Susurluk, A. 2008. Potential of the entomopathogenic nematodes *Steinernema feltiae, S. weideri* and *Heterorhabditis bacteriophora* for the biological control of the sugar beet weevil *Bothynoderes punctiventris* (Coleoptera: Curculionidae). J. Pest. Sci. 81(2008):221-225.

Figures

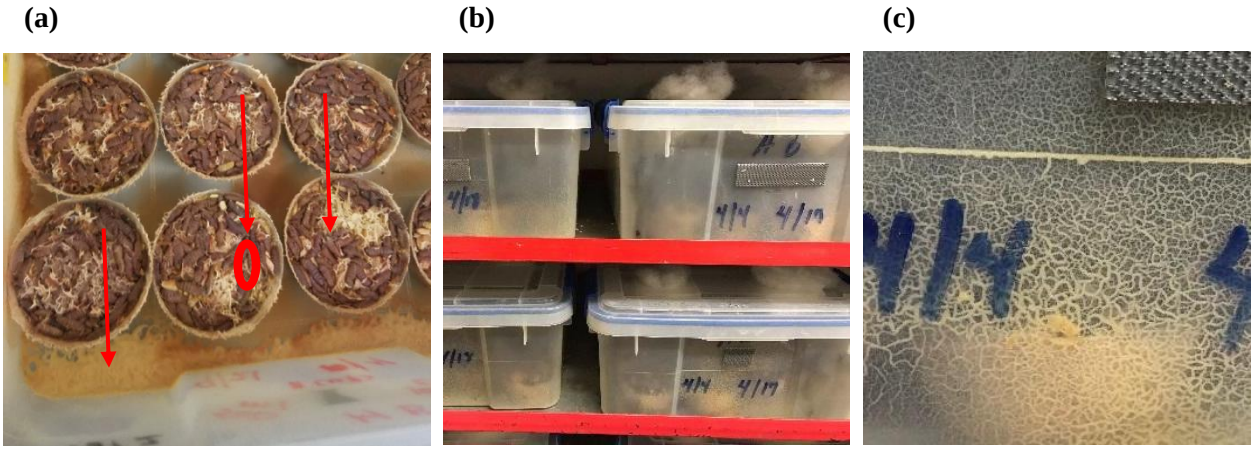

Fig. 2-1. Incubation environment and infectious juveniles (IJ) emergence and harvest conditions/density. **(a)** day of extraction with host cadavers and substrate mix in 147 ml clear PET cups surrounded by layers of IJs indicated by red arrows and circle **(b)** 25 L sealed plastic containers on harvest day and **(c)** visual representation of nematode emergence, travel and density once emerged from the host on the sides of the containers.

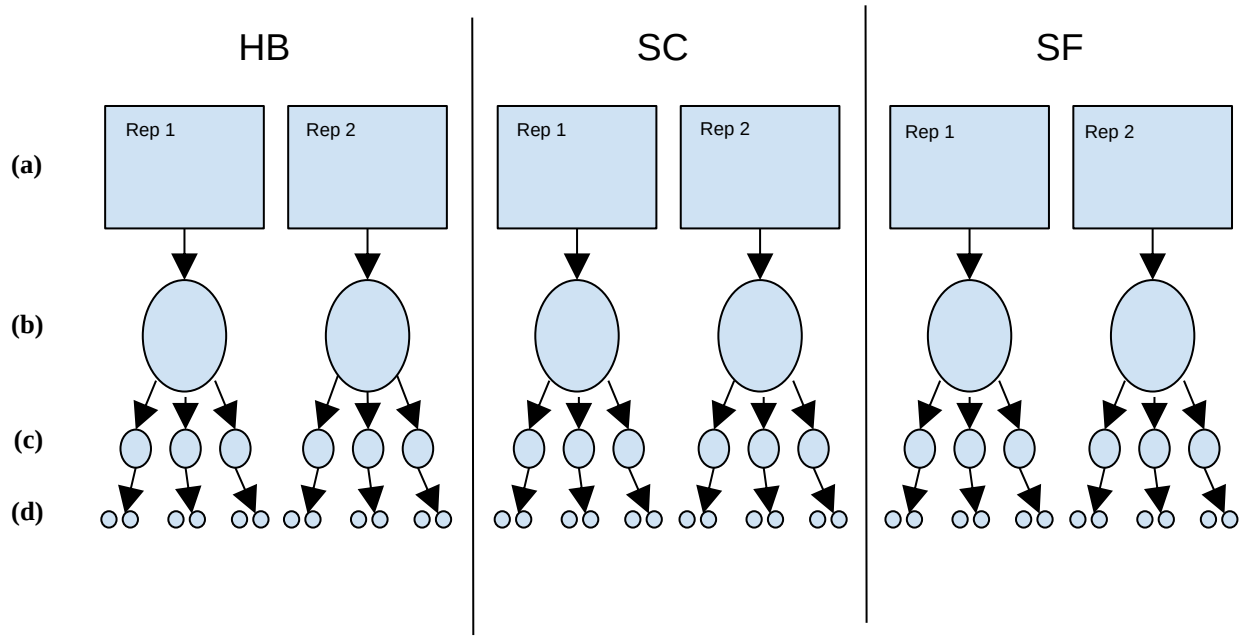

Fig. 2-2 Experiment sampling process for *Heterorhabditis bacteriophora* (HB), *Steinernema carpocapsae* (SC), and *Steinernema feltiae* (SF). (a) 25 L incubation container (b) 37.8 L bucket (c) 250 ml aliquot (d) two 25 µl sample per aliquot, n=6.

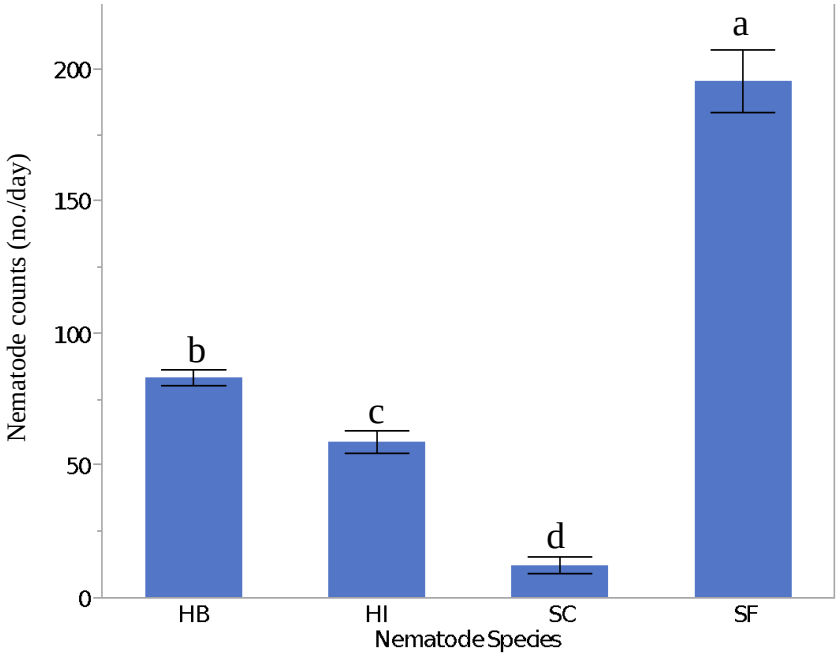

Figure 2-3. Average daily counts of infectious juveniles of each species, *Heterorhabditis bacteriophora* (HB), *Heterorhabditis indica* (HI) *Steinernema carpocapsae* (SC), and *Steinernema feltiae* (SF), over 5 days of cold storage at 4°C. Each error bar is constructed using a 95% confidence interval of the mean. Linear mixed model with repeated measures ANOVA (with an autoregressive one covariance structure) was used.

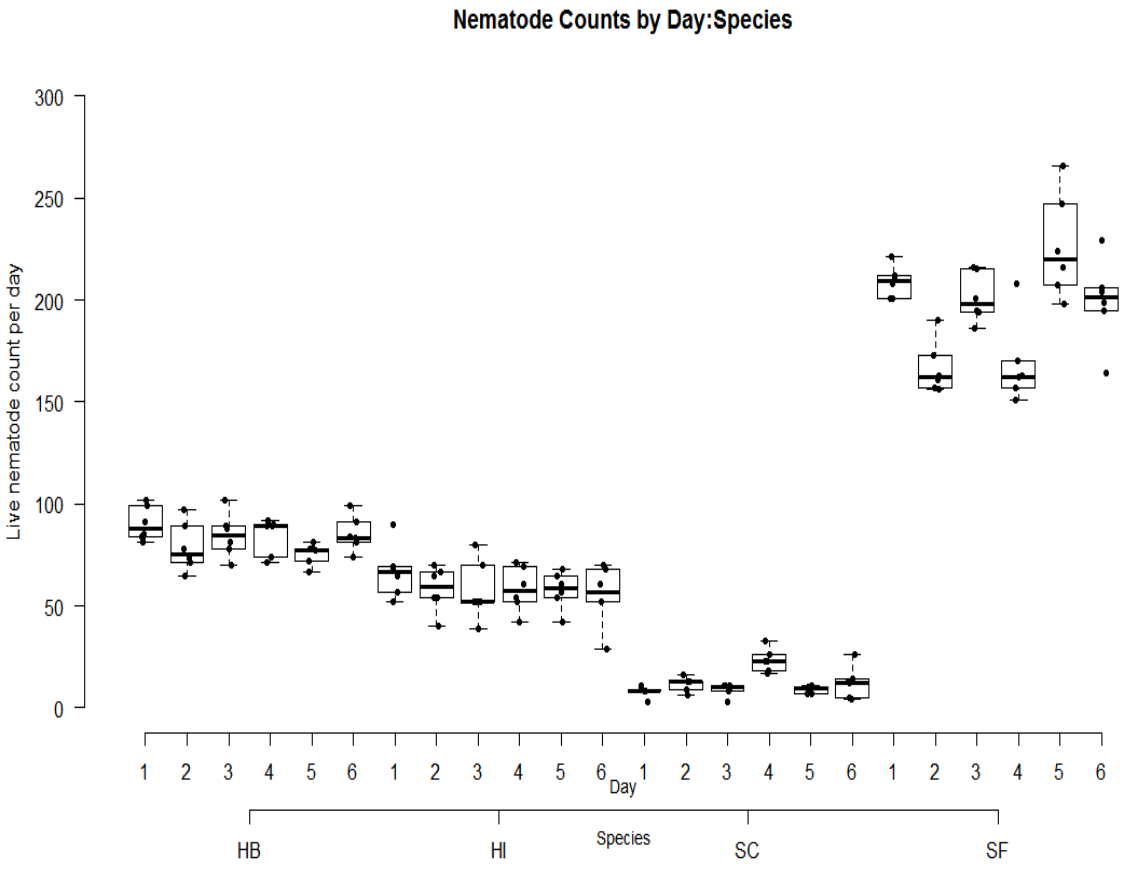

Figure 2-4. Effect of storage on infectious juvenile count per day for each species of nematode: *Heterorhabditis bacteriophora* (HB), *Heterorhabditis indica* (HI) *Steinernema carpocapsae* (SC), and *Steinernema feltiae* (SF). Each day is a comparative boxplot of the average daily counts collected across all three replications of this experiment. Storage temperature in a cooler was held at a constant 4°C for 5 days.

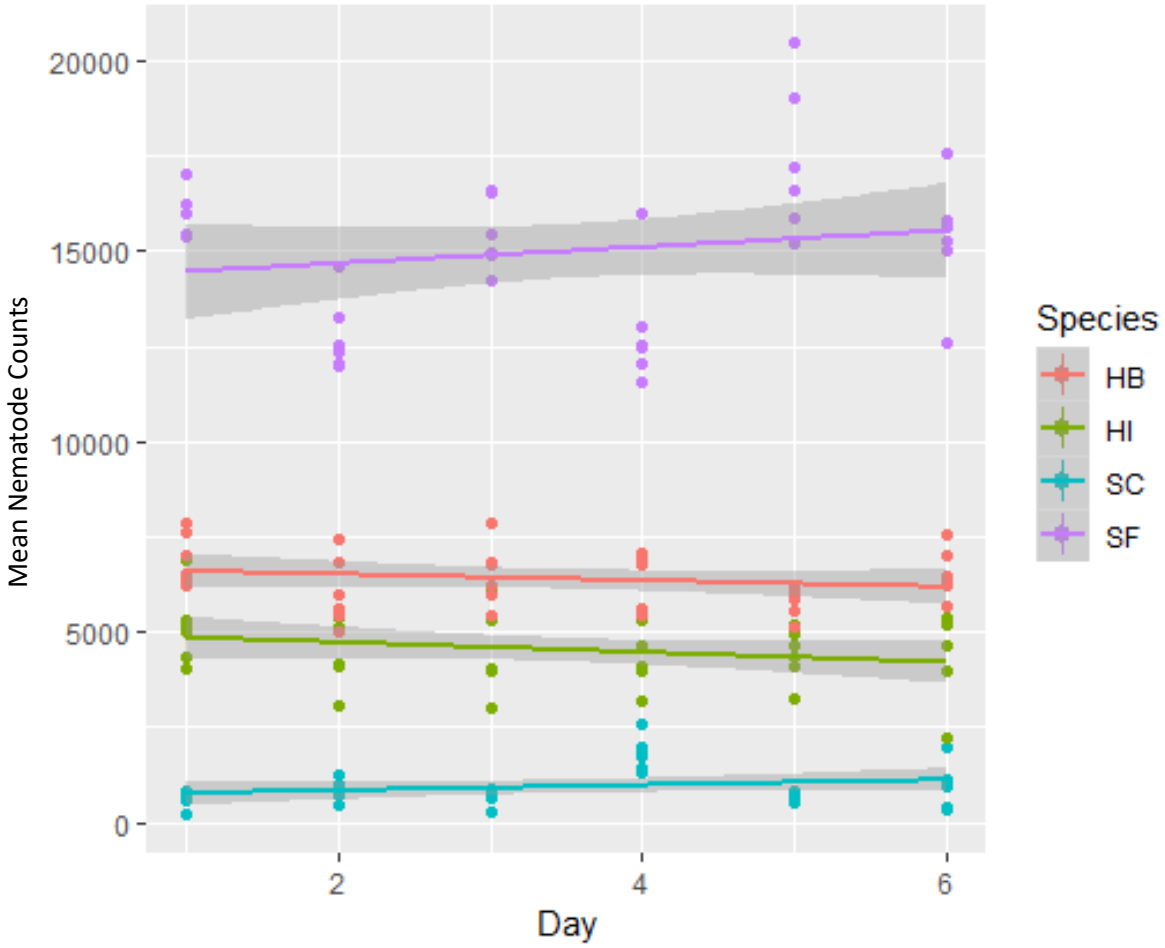

Figure 2-5. The mean nematode counts are shown for each species, *Steinernema feltiae, S. carpocapsae, Heterorhabditis bacteriophora and H. indica* for each sample day of each of the three replications (n=6) displayed as a trend line on a scatter plot. The line plotted represents the predicted linear model where we regressed average sample count (y) on day (x). There is a positive slope of R-squared at 0.947 for *Steinernema feltiae* (R Core Team, 2013).

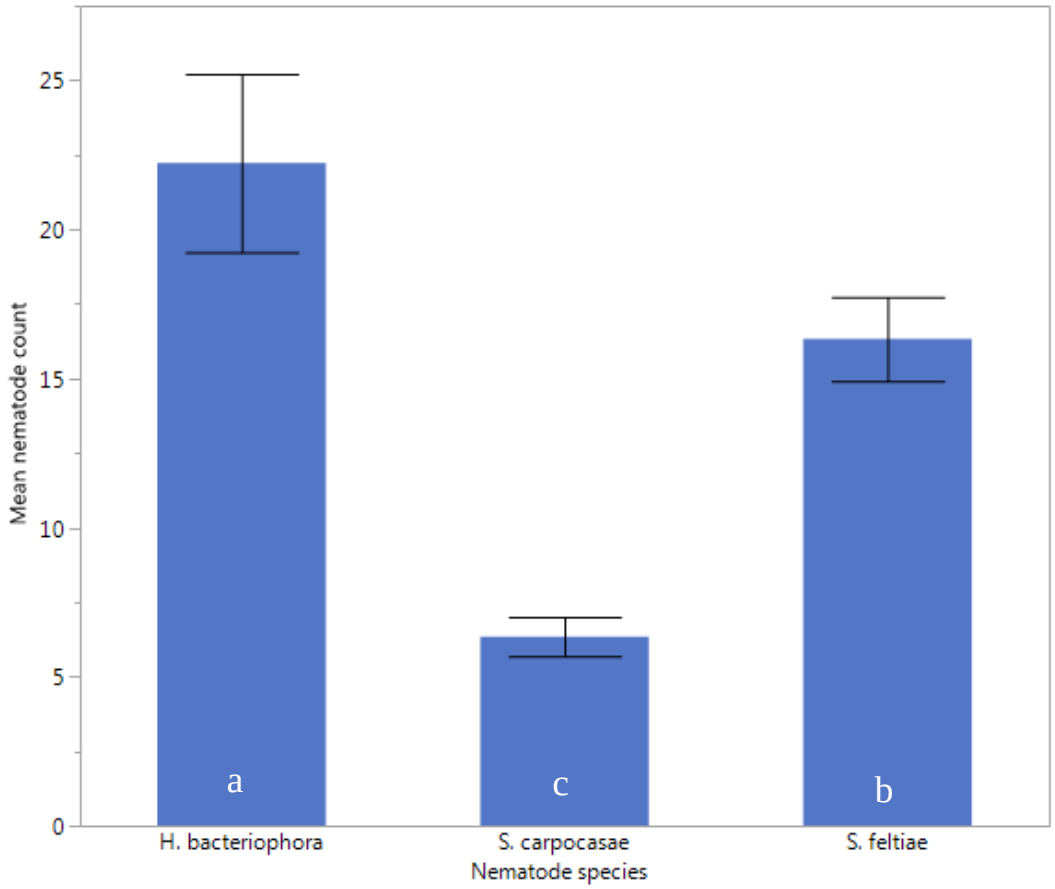

Figure 2-6. Mean daily infectious juvenile counts of *Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, and *Steinernema feltiae* over a 7-days period (DAE) in sealed 25 L incubation containers kept at a constant temperature of 22°C. Each error bar is constructed using a 95% confidence interval and letters indicate differences in counts per species using Student's t-test. Letters indicate significant differences between days by a one-way analysis through a connecting letters report.

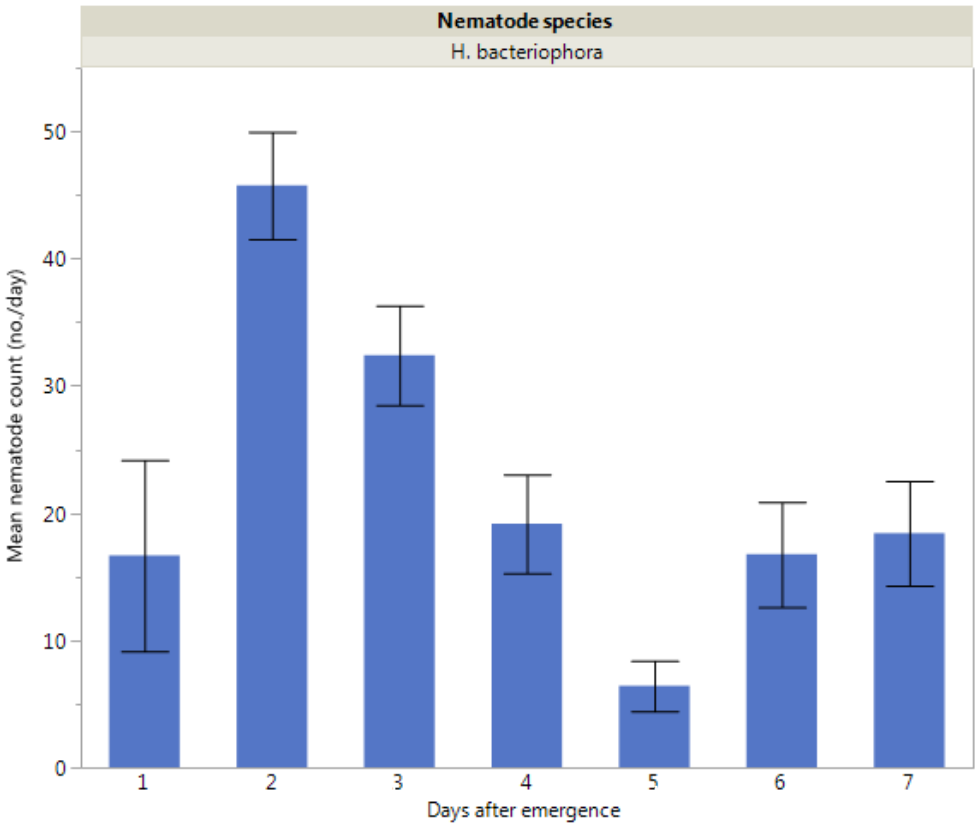

Figure 2-7. Mean daily infectious juveniles counts of *Heterorhabditis bacteriophora* (HB) over a 7-day emergence period in sealed 25 L incubation containers kept at a constant temperature of 22°C. Days shown indicate days from initial emergence through 7-day period. Each error bar is constructed using a 95% confidence interval and letters indicate differences in counts per day using Student's t-test. Letters indicate significant differences between days by a one-way analysis through a connecting letters report.

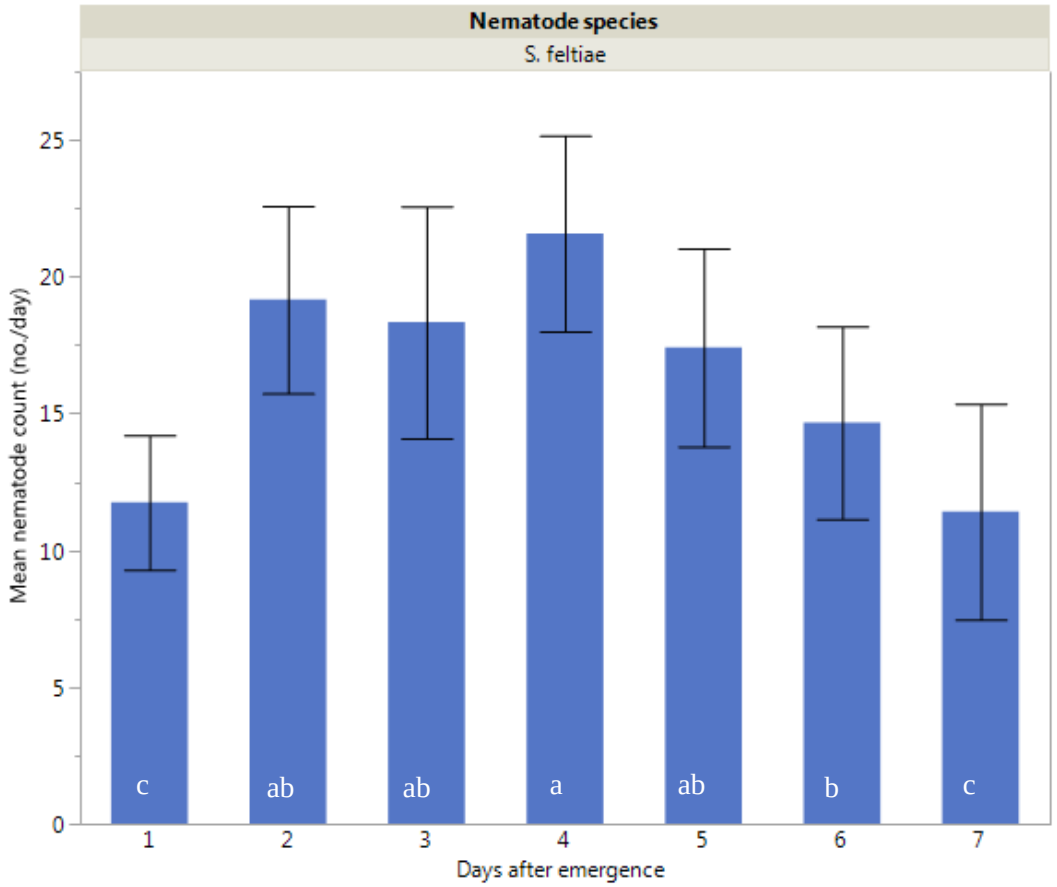

Figure 2-8. Mean daily infectious juveniles counts of *Steinernema feltiae* (SF) over a 7-day emergence period in sealed 25 L incubation containers kept at a constant temperature of 22°C. Days shown indicate days from initial emergence through 7-day period. Each error bar is constructed using a 95% confidence interval and letters indicate differences in counts per day using Student's t-test. Letters indicate significant differences between days by a one-way analysis through a connecting letters report.

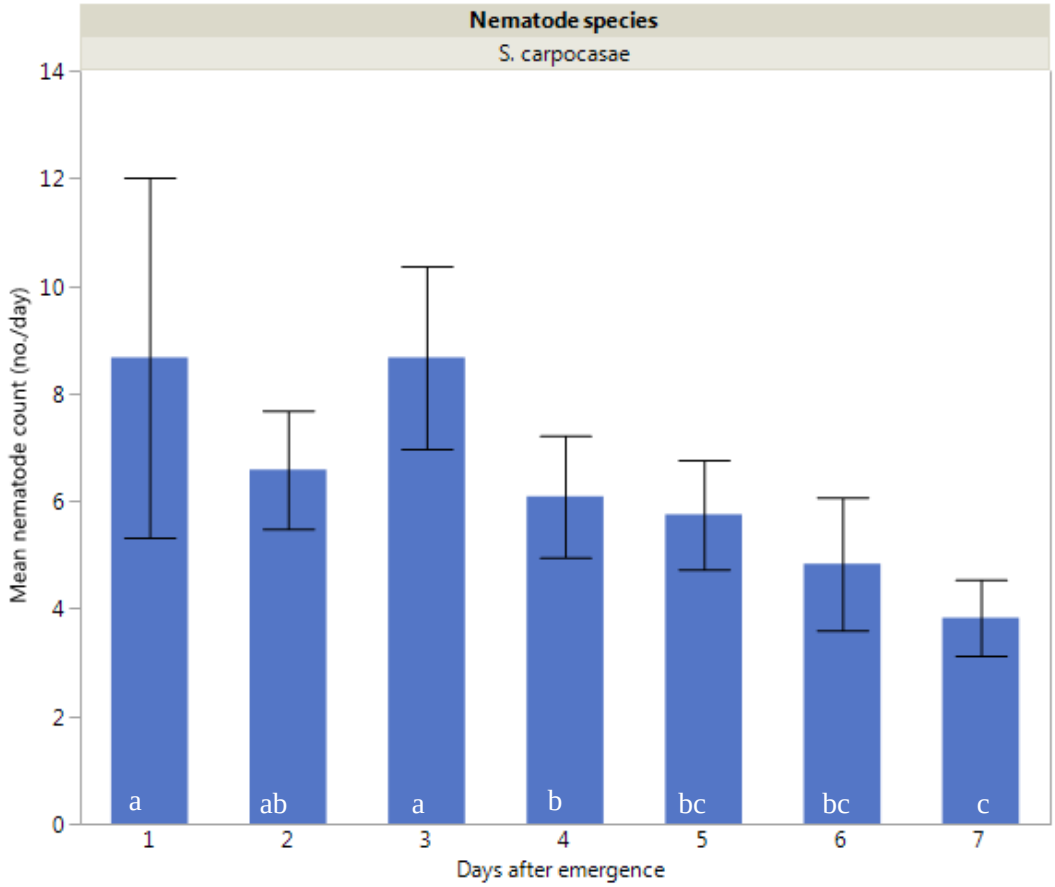

Figure 2-9. Mean daily infectious juveniles counts of *Steinernema carpocapsae* (SC) during the 7-day emergence in sealed 25 L incubation containers kept at a constant temperature of 22°C. Days shown indicate days from initial emergence through 7-day (workweek) period. Each error bar is constructed using a 95% confidence interval and letters indicate differences in counts per day using Student's t-test. Letters indicate significant differences between days by a one-way analysis through a connecting letters report.

Chapter 3: Control of *Botrytis cinerea* using ultraviolet-C radiation as an alternative to traditional fungicides.

Abstract

UV-C radiation is of major interest to industry crop production managers as an alternative to harsh fungicide use to prevent disease. UV-C has successfully been used in multiple other application types in other industries to satisfy similar objectives, such as the medical industry and water purification industry to sterilize liquids and surfaces. This study tested the efficacy of UV-C radiation as an alternative control to traditional fungicides to deactivate *B. cinerea* in vitro and to determine plant tolerance to UV-C. The crops tested were poinsettia (*Euphorbia pulcherrima*) and primula (*Primula vulgaris).* All the UV-C doses, 1.0, 2.8, 3.7 or 4 W/m^2, significantly decreased *B. cinerea* conidial germination in vitro and resulted in zero percent damage on poinsettia bracts. However, all UV-C doses during both replications caused minor damage, 15% or less, to primula flowers.

Introduction

Botrytis spp. are ubiquitous fungi with a necrotrophic lifestyle i.e., pathogens that attack hosts and feed on dead plant cells (Elad et al., 2007; Williamson et al., 2007). *Botrytis cinerea* is one of the most problematic species of this genus and causes damage on over 200 dicotyledonous species and dozens of monocotyledonous species (Williamson et al., 2007). These range from ornamental potted crops and cut flowers to agricultural crops and other plant hosts that fall victim to this pathogen (Williamson et al., 2007). *Botrytis cinerea* causes $10 to $100 billion in losses annually and many populations have become resistant to a broad range of fungicides. This species of *Botrytis* is the most studied necrotrophic plant pathogen affecting agricultural crops (Watkinson et al., 2016).

Botrytis cinerea produces a wide range of compounds including multiple metabolites that cause cell death (Aktaruzzaman et al., 2017). *Botrytis* spp. cause hypersensitivity once primary infection has occurred through the penetration of the cuticle layer (Watkinson et al., 2016). Hypersensitive response (HR) is a universal defense response by plants to fungal, bacterial and viral pathogen infection (Goodman and Novacky, 1994). This defense response occurs immediately after or during primary infection and causes rapid localized cell death around the infected cells. This plant defense response is very beneficial against biotrophic pathogens which are contained by HR but accelerates the growth and infection of necrotrophs like *B. cinerea* which feed on dead cells cased by HR (Watkinson et al., 2016).

Botrytis cinerea can survive and overwinter in temperatures as low as 0°C but is active between 2°C and 30°C (Elad et al., 2007). However, *B. cinerea* progresses through the different growth stages at temperatures between 12°C and 30°C with the most virulent growth stage producing conidia which germinate and infect plant cells. Growth initiates from sclerotia which germinate and produce mycelium which eventually produce conidia (Elad et al., 2007). Conidia are the primary stage of *B. cinerea* that infects host tissue after being easily dispersed by wind or water.

Traditional disease control consists of fungicides and cultural practices. *Botrytis cinerea* is known to have become resistant to many commercially available fungicides and is also known to continually adapt to new advances in fungicide technology (Ortuno et al., 2014). However, ultraviolet radiation has been shown to kill *Botrytis* spp. conidia (Braga et al., 2015). Ultraviolet (UV) radiation borders the visual spectrum between 200 to 400 nm. There are three ranges of UV radiation starting closest to the visual spectrum with UV-A (320-400 nm), UV-B (280-320 nm), then UV-C (200-280 nm) (McDonald, 2003). Ninety three percent of the solar radiation

that reaches the earth's surface is between 400 and 750 nm with the other 7% being the UV-A and UV-B spectrum between 295 nm to 400 nm; UV-C radiation does not reach the earth's surface (McDonald, 2003).

Ultraviolet radiation not only affects fungi but also causes ultrastructure cellular effects in most organisms. *Botrytis cinerea,* which causes grey mold on many harvested crops, can be quite sensitive to radiation in the UV spectrum (Darras et al., 2009). Direct exposure to UV-C radiation, which also is referred to as germicidal wavelengths, can be used to irradiate pathogens in combination with induction of host defenses (Darras et al., 2009). UV-C radiation can cause *B. cinerea* conidia to deactivate which will lessen severity of overall infection. A study conducted on cut flower freesia concluded that UV-C irradiation after artificial inoculation of *B. cinerea* on inflorescences resulted in lower disease severity than when UV-C was applied before artificial inoculation (Darras et al., 2009).

There have been many studies with UV-C for disease management that include many different species of fruit crops (Civello et al., 2006; Ippolito and Nigro, 2000; Stevens et al., 1997; Yaun et al., 2004). However, discussing a variety of studies requires an understanding of the units of measurement used in UV-C research. Ultraviolet radiation can be measured as irradiance (W/m^2/sec or mW/m^2/sec) or energy density (J/cm^2, mJ/cm^2 or kJ/cm^2) which incorporates time (seconds) and surface area in the measurement. This unit of measure (J/cm^2, mJ/cm^2 or kJ/cm^2) for a total dose is the combination of radiation density and total duration of exposure into one unit without the need to express total duration of the exposure. The dose can change based on the density of the radiation source (proximity to radiation) and/or the duration of the radiation applications, hence the duration length does not need to be noted in dosage units as long as the proper dose is conveyed.

UV-C induces disease resistance in carrots (*Daucus* spp.), citrus (*Citrus* spp.) and tomatoes (*Lycopersicum* spp.) through the accumulation of phytoalexins (Ouhibi et al., 2014). In a study using Romaine lettuce, *Lactuca sativa* L., applying UV-C (280 nm; 0.85 kJ/m^2) after harvest and before shipping significantly reduced the size of *B. cinerea* infected lesions on leaves for up to four days as compared to the control which received no UV-C exposure (Ouhibi et al., 2014). Another significant example of UV-C's efficacy is in its use on grapes during shipping where *Botrytis* spp. rots can cause high economic losses (Nigro et al., 1998). A study conducted in Italy applied UV-C to table grapes (*Vitis vinifera* L. cv. Italia), resulting in a significantly lower level of disease in berries that were inoculated with *B. cinerea* 24 hours after UV-C application compared to those inoculated just 10 minutes after UV-C application (Nigro et al., 1998). Even though it is known that UV can be very dangerous at high doses, altering DNA, causing mutations, as well as disturbing biological processes, UV can also be harnessed to prevent disease damage and ensure quality of plants and produce during postharvest conditions (Ouhibi et al., 2014).

Ornamental crops have similar disease pressures during production and postharvest transport. However, there has been little research on using UV-C for disease management on ornamental crops. We believe there to be significant disease management potential on ornamental crops similar to that of produce crops, as mentioned above. Our initial objective was to determine if brief exposures of UV-C doses can deactivate *Botrytis cinerea* conidia present on plant tissues of ornamental crops. We hypothesize that higher doses will deactivate *B. cinerea* completely but will damage the plant tissue while lower doses will partially deactivate conidia and not damage plant foliage nor petals. We also hypothesize that moisture presence during UV-C exposure and deactivation severity will have a negative confounding relationship. Meaning

that the more moisture present reflects or in some cases magnifies the UV-C being applied compared to when moisture is not present.

Materials and Methods

UV-C deactivation of *B. cinerea* in vitro

This portion of the study was to determine if UV-C can deactivate *B. cinerea* conidia when moisture is present in vitro with a water film present in petri plates. The presence of moisture can reduce the impact of UV because of the reflective properties of water and needs testing due to moisture being a key factor in the conducive environment for *Botrytis* spp. infection.

Rearing and plating inoculum.

Botrytis cinerea was isolated from an infected *Petunia x hybrida* petal and a clean isolate was grown on two 100 x 10 mm sterile petri plates containing full strength potato dextrose agar (PDA) in a lab at 20°C for two weeks. Once *B. cinerea* covered the plates and produced conidia, the conidia were harvested by adding distilled (DI) water first and then scraping growth out of PDA plates into a 500 ml beaker. The liquid concentrate was sieved through cheesecloth into a clean 500 ml beaker. Conidia concentration was determined through the use of a hemocytometer. We added 500 ml of DI water to create a liquid volume of 1000 ml of 10^4 conidia/ml. Conidial suspension was mixed thoroughly and 25 µl was taken and placed on a 35 x 10 mm plate with a maximum holding capacity of 10 ml and spin plated to disperse conidia over the plate. This technique was used for all plates in this study. Dry sample plates were left to sit in the laminar-flow hood for 10 minutes while moisture evaporated, leaving conidia dry and exposed. The wet samples were immediately subjected to UV-C applications.

UV-C applications.

A handheld UV-C module (Blade Unit, Part# HH-18-120T; American Ultraviolet, Hackettstown, NJ) was braced at the handle with a clamp and hung 12.7 cm above a 10-mm high petri plate (Figure 3-1). The module's precise distances above the plate and durations of exposure were based on preliminary tests prior to the experiment to ensure consistency and keeping the exposure duration within practical limits practicality of the application based on the dose duration. For safety, the module and the exposed areas were covered by Mylar with metallic coating reflective material (Vivosun, Denver, CO) to prevent radiation leakage outside of the target area. All UV-C exposures were conducted under a laminar flow hood in a lab with an average temperature of 20°C.

Ultraviolet radiation measurements were taken with an ILT 1000 data-logging light meter optometer (International Light Technologies, Peabody, MA). With the covered system described above, the UV-C module was turned off between replications and turned on to stabilize for 2 min before use. Specific UV-C doses were determined by maintaining the module at a fixed distance of 12.7 cm above the petri plate and varying the exposure time. Exposure times were 0.5, 1.0, 1.5 or 2.0 min corresponding to UV-C doses of 1.0, 2.8, 3.7 or 4.0 W/m^2. UV-C doses were not linear. Therefore, to reduce variability each treatment was conducted with the same method. Each treatment started with turning the UV-C module off and using the same stabilization rate for every application to ensure proper and consistent W/m^2 dose per treatment. Control plates were not exposed to UV-C. Plates were placed directly under the module, the lid of each plate was removed, and timer initiated for each replication of each dose. Once the dose was complete, the lid was placed back on top of the plate and the plate was sealed with parafilm to keep it sterile. There were four UV-C doses and an untreated control. For each dose there were two groups, wet or dry plates; each group was composed of four replications and a nontreated

control. Each replication was an individual spin-plated *B. cinerea* conidia plate. Deactivation data were taken as a percentage of germination where 100 conidia per plate were counted under a 10x magnification 24 hours after UV-C dose. Conidia were considered germinated if any germ tube was observed protruding out of the conidia. Means were compared using Student's t-test and were constructed through a connecting letters report.

Evaluation of plant tolerance to UV-C dose

The following study was conducted to evaluate plant tolerance (damage response) to UV-C doses. This study was conducted twice. The UV-C Blade module was mounted at 12.7 cm above the top of the plant canopy (Figure 3-2). UV-C was applied as described above using the same intervals as in the in-vitro petri plate study, 0.5, 1.0, 1.5, 2.0, or 5.0 min, corresponding to UV-C doses of 1.0, 2.8, 3.7 or 4.0 W/m^2 with control plants not exposed to UV-C. We included one extreme exposure of 5.0 min (10.1 W/cm^2) to ensure damage could be observed. Poinsettia (*Euphorbia pulcherrima*) and primula (*Primula vulgaris)* were grown in a commercial greenhouse (Battlefield Farms, Rapidan, VA). Poinsettia plants were grown in individual 2.4-L pots and primula plants were grown in 1.2-L pots. There were five individual poinsettia plants per dose per study replication. Poinsettia plants were all the same red cultivar, Christmas Day (Figure 3-3). Poinsettia plants were cleaned of damaged and yellowing bracts and clean remaining bracts were counted. Primula plants were composed of a mixture of six colors (pink bi-color, blue, red with yellow eye, pink with yellow eye, white with yellow eye, and yellow) in an industry series, Primera^{TM} mix (Figure 3-4). Primula plants were cleaned of old or damaged flowers before exposure to UV-C and clean flowers counted. Six primula plants, one of each color were exposed to the UV-C doses. After UV-C exposure, plants were immediately placed on tables in a shaded greenhouse. UV-C damage was assessed 24 hours after exposure as a

percentage of damaged poinsettia bracts or primula petals that had bleached or darkened in color compared to the clean bract and petal count before exposure (Figure 3-5). The damage data were taken as a percentage of the number of bracts or petals damaged compared against clean counts before treatments. Data were subjected to one-way ANOVA and means were separated using Student's t-test.

Plant inoculation, UV-C exposure and shipping simulation

The following experiment was conducted to observe the effectiveness of UV-C as a management tool for controlling *Botrytis cinerea* infection on ornamental crops under shipping conditions. Based on the results from the studies above to ensure proper methods for a combined experiment, *B. cinerea* was cultured in vitro as described above to use as inoculum. Using the same cultivars as above, poinsettia and primula plants were grown in a commercial greenhouse (Battlefield Farms Inc.). Plants were grown to desired color and height requirements and inoculated with *B. cinerea* as a liquid spray (10^4 conidia/ml) on poinsettia bracts and primula flowers. Immediately after the inoculation event, while still wet, plants were subjected to UV-C doses as described above, 1.0, 2.8, 3.7 or 4.0 W/m^2. Plants were left wet from artificial-inoculation spray to promote a conducive environment for *B. cinerea* infection. Immediately after UV-C exposure, poinsettia plants were placed on carts which were wrapped with plastic to mimic shipping protocols (Figure 3-6). Primula plants were placed in cardboard boxes which were sealed and placed on carts. Plants were stored in a shipping trailer to mimic shipping environment for 24 hours at a constant temperature of 14^0C and 70% relative humidity (RH). There were five single plant replications for each crop arranged in a randomized design per box for primula and per shelf per cart for poinsettia. After 24 hours of simulated shipping storage, plants were taken from the shipping trailer and placed in a shaded greenhouse environment.

Botrytis cinerea damage was assessed 24 hours after the end of the shipping simulation. Damage was defined as visible *B. cinerea* infection described as black and grey lesions (Figure 3-7), or as UV-C damage, characterized by bleaching on poinsettia bracts or primula petals (Figure 3-8). Only poinsettia bracts that were infected by visible lesions were counted per plant and only flowers that were infected were counted per primula plant. Percent damage was calculated as the percentage of damaged bracts or flowers compared to clean counts before the simulation. Data were subjected to one-way ANOVA and means were separated using Student's t-test (R Core Team, 2013).

Results

UV-C deactivation of *B. cinerea* in vitro

UV-C deactivated *Botrytis cinerea* conidia in vitro (Figure 3-9). There was no significant difference in *B. cinerea* conidia germination between wet (W) and dry (D) plates after exposure. Our study showed that any of the UV-C doses, 1.0, 2.8, 3.7 or 4 W/m^2, significantly decreased *B. cinerea* conidial germination after exposure when compared to the non-exposed control. The non-exposed control was fully colonized while the UV-C treated plates had very few if any germination tubes present.

Evaluation of plant tolerance to UV-C dose

Poinsettia. The lower doses of UV-C exposure did not cause notable damage on poinsettia bracts. The percent damage on poinsettia bracts was consistently 0% through the doses, 1.0, 2.8, 3.7 or 4 W/m^2 and non-treated control. However, the UV-C dose of 10.1 W/m^2 caused significant damage and the bracts on the poinsettia plants had visible symptoms of bleaching and darkening of bract color (Figure 3-10).

Primula. All UV-C doses during both replications caused minor damage, 15% or less, to primula flowers (Figure 3-11). Both runs of the experiment were consistent with respect to effects of UV-C exposure. Therefore, data were pooled. Damage caused by the dose of 1.0 W/m^2 was not significantly different from that of either 2.8 W/m^2 or control. The damage was significantly different between doses of 2.8, 3.7 or 4.0 W/m^2 and the non-exposed control. Primula petal color and percent damage, among both replications and all doses were significantly different except when comparing 1.0 W/m^2 to the control. The UV-C dose of 10.1 W/m^2 had similar damage to that of poinsettia with severe bleaching (data not presented).

Plant inoculation, UV-C exposure and shipping simulation

During the shipping simulation, both genera exhibited a slight decline in overall visual quality due to the harsh environmental conditions. The combined effect of UV-C, moisture residue, *B. cinerea* and shipping environment negatively impacted plant quality. UV-C, at all doses, lessened the infection of *B. cinerea* , (data not shown) but increased the bleaching response, perhaps from the interaction of UV-C and moisture residue on plant tissue. Poinsettia bracts, on average, were less damaged than primula petals.

Poinsettia.

The control plants infected with *B. cinerea* exhibited black speckling and black spotted lesions and large black lesion dieback (Figure 3-12a). The UV-C-treated plants were consistent in their response with black small infection areas covered by large, bleached circles around the area to suggest an interaction between UV-C exposure, moisture residue and *B. cinerea* infection on plant tissues (Figure 3-12b). The lesions were larger and observed damage brighter than on non-UV-treated plants? with white circles around the droplets that held the *B. cinerea* conidia along

with large white or bleached areas where droplets of moisture would puddle at the end of petals. All plants responded negatively to the combination of UV-C in the presence of *B. cinerea* with 3.7 W/m^2 causing the most damage (Figure 3-13).

Primula. The control plants were infected with *B. cinerea* and showed symptoms similar to those on poinsettia with black speckling and black spotted lesions (Figure 3-12c). Also, as shown in Figure 3-5, primula petals easily bleached at doses of 3.7 W/m^2 or greater. . Damage percentages on plants subjected to treatments 3.7 and 4 W/m^2 were higher, numerically, and flowers had slightly more visible damage than seen on the control plants (Figure 3-14). However, these differences were not significant. Treatments 1 and 2.8 W/m^2 resulted in a lower level of plant damage compared to the control plants, numerically and visually, but differences were not statistically significant. The percent damage assessed on the control plants was not significantly different from any of the UV-C treated primula plants However, primula flowers treated with 1 W/m^2 had significantly lower damage as compared to flowers subjected to treatments 3.7 and 4 W/m^2.

Discussion

For a greenhouse producer, understanding the relationships between technology, the environment and pests are key to properly managing pests and plant quality during all facets of the supply chain. Some horticultural and agricultural markets have used UV-B and -C radiation in the past to induce crop resistance to fungal infection, to deactivate pathogens on contact, to increase germicidal effectiveness in combination with temperature, and to increase fruit phytonutrient content (Rouphael et al., 2018). In addition to fungal pathogens, bacterial pathogens such as soft rot (*Pseudomonas spp.*) can cause significant crop damage and losses similar to *Botrytis* spp. (Escalona et al., 2010). UV-C has been tested on bacterial pathogens using baby spinach, where

the authors concluded that disease symptoms were reduced with exposure to UV-C at the beginning of storage (Escalona et al., 2010). Using UV radiation has proven successful, but the practical implementation of this tool is challenging due to the hormetic impact UV can have, not only on crops when used improperly, but also on humans (Shama, 2007). Studies were conducted using temperature, UV-C, and white light to deactivate *B. cinerea* and *Monilia fructigena* on strawberries and sweet cherries (Marquenie, 2003). These studies used different intervals of pulsed white light with different durations of high temperatures in combination with differing doses of UV-C (Marquenie et al., 2002; Marquenie et al., 2003). Marquenie et al. (2003) concluded there was an increase in deactivation of both pathogens due to the combined effect synergy of these three control measures when applied together. Another study applied both UV-C and the biological control yeast, *Cryptococcus laurentii*, for control of postharvest disease on tomato fruit (Zhang et al., 2013). This study used these two control measures together to determine whether the combined synergy of these could inhibit further spread of *B. cinerea* infection of already decaying fruit. Zhang et al. (2013) concluded that together UV-C and *Cryptococcus laurentii* were effective at inhibiting further decay caused by *B. cinerea*. Similar to the approaches of other studies, Veazie et al. (2007) also used UV-C to deactivate *B. cinerea* on blueberries; however, during his experiment they concluded that the use of UV-C could not only reduce disease severity but also provoke accumulation of higher concentrations of desired phytonutrients like anthocyanins.

Whereas many of these studies proved to be successful on produce, fruits and vegetables, there has been very little work done on the efficacy of UV-C on ornamental crops and on flower petals, specifically. Darras et al. (2010) conducted a study using the cut flower freesia, because this crop's flowers have significant losses due to petal speckling caused by *B. cinerea* during

transport. UV-C was used on freesia petals to reduce *B. cinerea* infection and was successful at reducing lesion number and diameter by 24% (Darras et al., 2010). This was not very promising as this approach needs to have a higher efficacy in order to be practically useful. From the results in both the in-vitro study and shipping simulation, we determined that UV-C, within the range of our four doses, whether dry or in the presence of a water film reduce Botrytis cinerea infection supports the effective approach from Darras et al. (2010).

When evaluating plant tolerance to UV-C, different vegetative and reproductive plant tissues could be impacted differently (Kakani, 2003). We determined that flower petals of primula are more sensitive to UV-C exposure than the bracts of poinsettia (Figure 3-11). Overall, the results from our in-vitro study and plant tolerance study suggested UV-C may be applied with low doses incurring minor to no damage depending on the type of the plant. The next practical step was to apply them together through our *B. cinerea* inoculation, UV-C exposure, and shipping simulation study. During this study we determined that both plants responded negatively to UV-C in the presence of moisture. A constant temperature of 14^0C and 70% relative humidity is known to facilitate *B. cinerea* infection inside the tractor-trailer (Yu and Sutton, 1998).

The results varied in UV-C damage severity between the two species under the same conditions. Primula flowers were found to be more sensitive to UV-C than poinsettia bracts, with more noticeable damage after exposure. Even though there were fewer lesions on the UV-C exposed poinsettia than primula plants, the damage that was noted on poinsettia bracts was obvious and considered unacceptable. The damage on primula plants treated with the higher dosages tended to be greater than that noted on the control plants (difference not significant), suggesting that our control measures at high doses caused more damage than the combination of

the fungi and the conducive environment. Poinsettia had similar results, but the damage was not as great. Poinsettia bracts only showed obvious damage at higher doses (3.7 and 4.0 W/cm^2). Even at these higher doses the extent of damage was still lower than that observed on primula at the lowest dose of (1.0 W/m^2).

Ultraviolet radiation-C has strong germicidal ability directly on pathogens but the difference in exposure required to kill the pathogen and what the plants can tolerate makes the use of UV-C for *B. cinerea* control on ornamental plants very difficult to implement. However, we can conclude that different plant tissue types (foliage or petals) may respond differently to similar exposures. With these conclusions there may be more practical approaches to control *B. cinerea* with UV-C but an obvious first step if using UV-C, based on the data presented, would be to ship plants that are dry.

Literature Cited.

Aktaruzzaman, M.D., T. Afroz, S. Hong and B. Kim. 2017. Identification of *Botrytis cinerea*, the cause of post-harvest gray mold on broccoli in Korea. Research in Plant Disease 23(4):372-378.

Braga, G.U.L., D. E.N. Rangel, E. K.K. Fernandes, S. D. Flint and D. W. Roberts. 2015. Molecular and physiological effects of environmental UV radiation on fungal conidia. Curr Genet 61(2015):405-425.

Civello, P.M., A.R. Vicente and G.A. Martinez. 2006. UV-C technology to control postharvest diseases of fruits and vegetables. Transworld Research Network.

Darras, A.I., D.C. Joyce and L.A. Terry. 2009. Postharvest UV-C irradiation on cut *Freesia hybrid* L. inflorescences suppresses petal specking caused by *Botrytis cinerea.* Postharvest Biol. Technol. 55(2010):186-188.

Elad, Y., B. Williamson, P. Tudzynski and N. Delen. 2007. *Botrytis* spp. and diseases they cause in agricultural systems – An introduction, p. 1-8. In: Elad, Y., B. Williamson, P. Tudzynski and N. Delen (eds.). *Botrytis:* Biology, Pathology and Control. Springer, Dordrecht, The Netherlands.

Escalona, V.H., E. Aguayo, G.B. Martinez-Hernandez and F. Artes. 2010. UV-C doses to reduce pathogen spoilage bacterial growth *in vitro* and in baby spinach. Postharvest Biology and Technology 56(2010):223-231.

Fernández-Ortuño, D., A. Grabke, X. Li and G. Schnabel. 2014. Independent emergence of resistance to seven chemical classes of fungicides in *Botrytis cinerea*. Phytopathology 105:424-432.

Goodman, R.N. and A.J. Novacky. 1994. The hypersensitive reaction in plants to pathogens: A resistance phenomenon. APS Press, St Paul, MN.

Ippolito, A., and F. Nigro. 2000. Impact of preharvest application of biological control agents on postharvest disease of fresh fruits and vegetables. Crop Protec. 19(2000):715-723.

Kakani, V.G., K.R. Reddy, D. Zhao and A.R. Mohammed. 2003. Effects of Ultraviolet-B Radiation on Cotton (*Gossypium hirsutum* L.) Morphology and Anatomy.

Marguenie, D., C.W. Michiels, J.F. Van Impe., E. Schrevens and B.N. Nicolai. 2002. Pulsed White lighting combination with UV-C and heat to reduce storage rot of strawberry. Postharvest Biol. and Technol. 28(2003):455-461.

Marquenie, D., A.H. Geeraerd, J. Lammertyn, C. Soontjens, J.F. Van Impe, C.W. Michiels and B.M. Nicolai. 2003. Combination of pulsed white light and UV-C or mild heat treatment to inactivate conidia of *Botrytis cinerea* and *Monilia fructigena.* Food Microbiol. 85(2003):185-196.

Mc Donald, M.S. 2003. Photomorphogenesis, p. 221-223. Photobiology of Higher Plants. John Wiley & Sons Inc., Hoboken, NJ.

Nigro, F., A. Ippolito, G. Lima. 1998. Use of UV-C light to reduce *Botrytis* storage rot of table grapes. Postharvest Biology and Technology 13(1998):171-181.

Ouhibi, C., H. Attia, P. Nicot, L. Urban, M. Lachaal and J. Aarrouf. 2014. Effect of UV-C radiation on resistance of romaine lettuce (*Lactuca sativa* L.) against *Botrytis cinerea* and *Sclerotinia minor.* J. Phytopathol. 163(2015):578-582.

Rouphael, Y., M.C. Kyriacou, S.A. Petropoulos, S.D. Pascale and G. Colla. 2018. Improving vegetable quality in controlled environments. Scient. Hort. 234(2018):275-289.

Shama, G. 2007. Process challenges in applying low dose of ultraviolet light to fresh produce for eliciting beneficial hermetic responses. Postharvest Biol. and Technol. 44(2007):1-8.

Stevens, C., V.A. Khan, J.Y. Lu, C.L. Wilson, P.L. Pusey, M.K. Kabwe, E.C.K. Igwegbe, E. Chalutz and S. Droby. 1998. The germicidal and hermetic effects of UV-C light on reducing brown rot disease and yeast microflora of peaches. Crop Protec. 17(1998):75-84

Veazie, P.P., J.K. Collins and L. Howard. 2007. Blueberry fruit response to postharvest application of ultraviolet radiation. Postharvest Biol. Technol. 47(2008):280-285.

Watkinson, S.C., L. Boddy and N.P. Money. 2016. Pathogens of Autotrophs, p. 276-277. The Fungi, third edition. Academic Press, San Diego, CA.

Williamson, B., B. Tudzynski, P. Tudzynski and J. A. L. Van Kan. 2007. *Botrytis cinerea*: the cause of grey mold disease. Molecular Plant Pathology 8(5)561-580.

Yaun, B.R., S.S. Summer, J.D. Eifert and J.E. Marcy. 2004. Inhibition of pathogens on fresh produce by ultraviolet energy. Food Microbiol. 90(2004):1-8.

Yu, Hai and C. Sutton. 1989. Effects of inoculum density, wetness duration, and temperature on control of *Botrytis cinerea* by *Gliocladium roseum* in raspberry. Canadian Journal of Plant Pathol. 20:243-252.

Zhang, C., K. Chen and G. Wang. 2013. Combination of the biocontrol yeast *Cryptococcus laurentii* with UV-C treatment for control of postharvest disease of tomato fruit. BioControl 58:269-281.

Figures

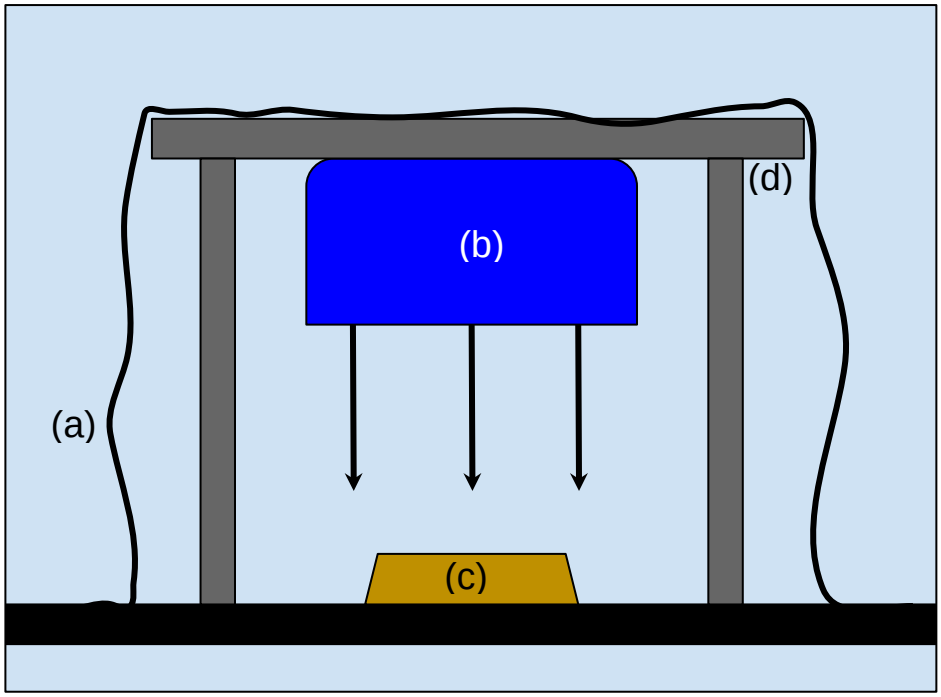

Fig. 3-1 Visual representation of the mount and positioning of the UV-C module in relation to a petri plate. (a) Mylar reflective plastic, (b) UV-C Blade module, (c) petri plate, (d) structure mount.

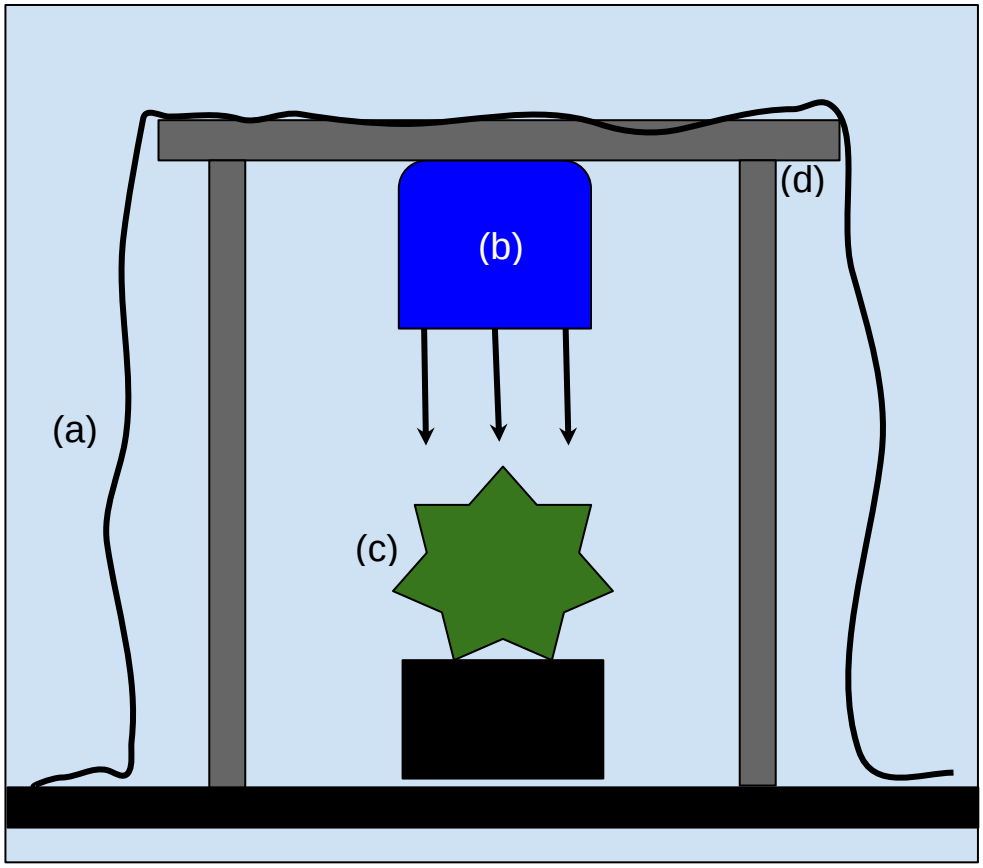

Fig. 3-2 Visual representation of the mount and positioning of the UV-C module in relation to a plant. (a) Mylar cloth covering, (b) UV-C Blade module, (c) potted plant, (d) structure mount.

Fig.3- 3 First replication of plant tolerance study of Poinsettia (*Euphorbia pulcherrima*) 'Christmas Beauty' before UV-C exposure.

Fig. 3-4 First replication of plant tolerance study of primula (*Primula vulgaris)* before UV-C exposure.

Fig. 3-5 Primula (*Primula vulgaris)* flower showing damage from UV-C dose (3.7 W/m^2) with bleached lines on petals.

Fig. 3-6 Trailer with both poinsettia (*Euphorbia pulcherrima*) on the plastic wrapped cart and primula (*Primula vulgaris*) in boxes on carts before shipping simulation.

Fig. 3-7 Poinsettia bracts showing damage (black lesions) from infection of *Botrytis cinerea* after artificial inoculation.

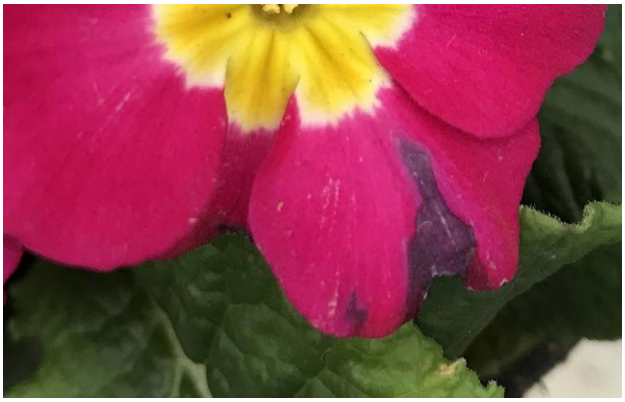

Fig. 3-8 Primula (*Primula vulgaris)* flower showing damage (black lesion) on flower petal from infection of *Botrytis cinerea*.

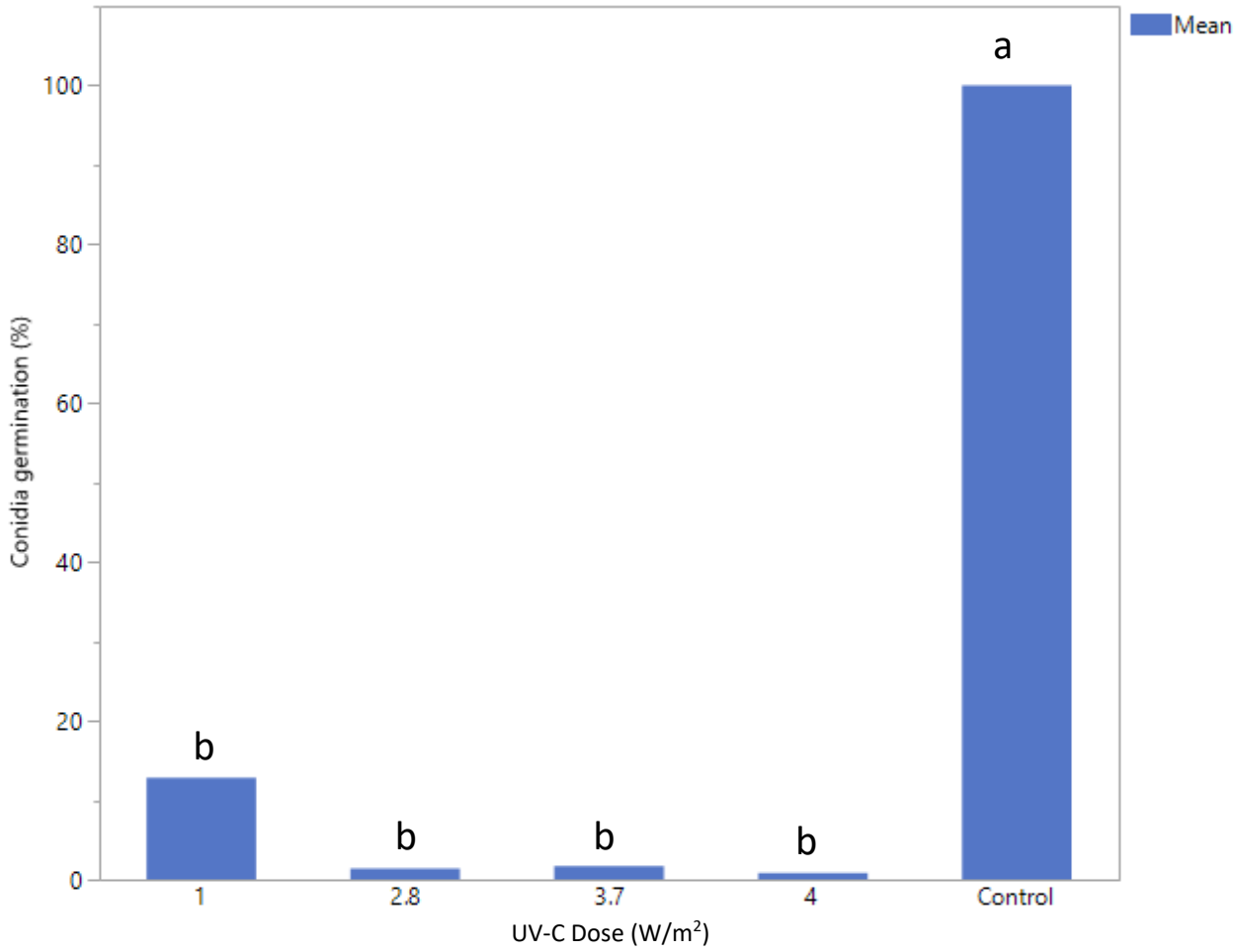

Fig. 3-9 Mean percent germination of *Botrytis cinerea* conidia on petri plates after UV-C application at four doses compared to a non-exposed control. Letters indicate significant differences in mean comparisons using Student's t-test and were constructed through a connecting letters report.

Fig. 3-10 Poinsettia (*Euphorbia pulcherrima*) 24 hours after exposure to UV-C at 10.1 W/m^2. Damage observed was darkening and bleaching of bract color on exposed areas.

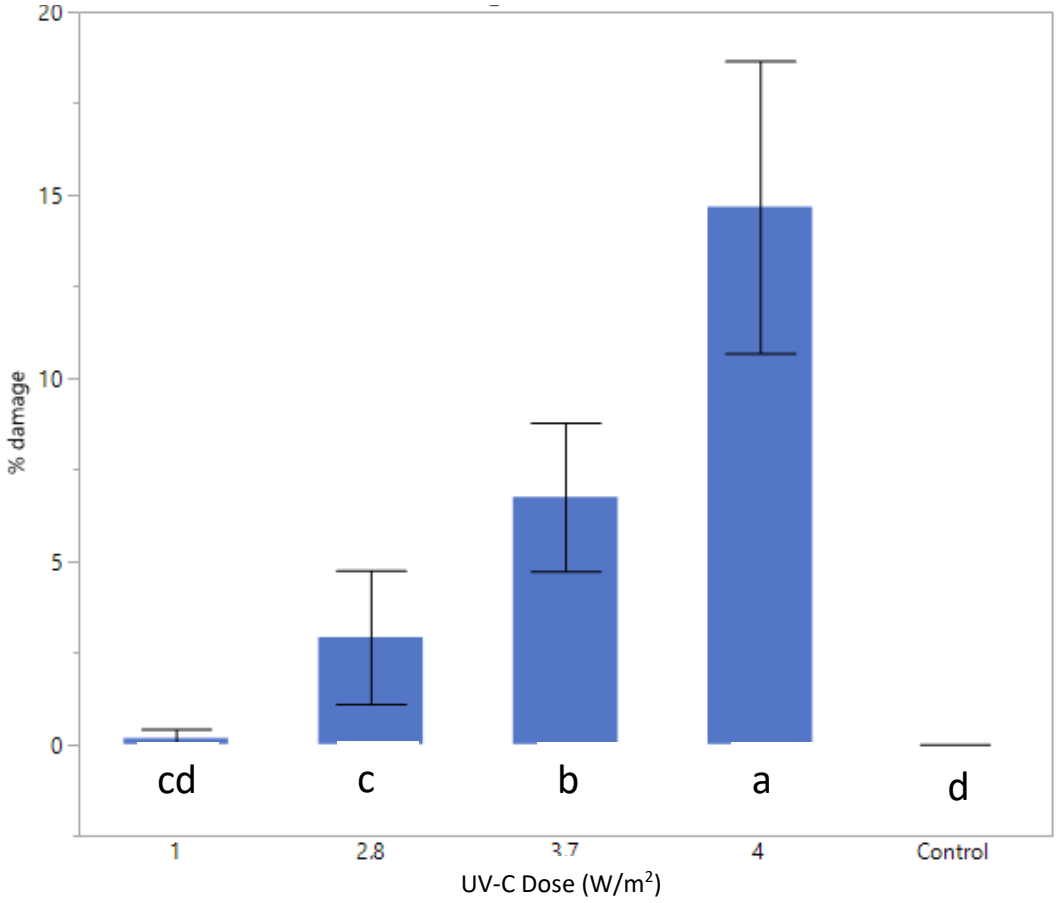

Fig. 3-11 Mean percent damage on primula flowers 24 hours after UV-C dose. Error bar is constructed using a 95% confidence interval. Letters indicate significant differences in mean comparisons through Student's t-test and were constructed through a connecting letters report.

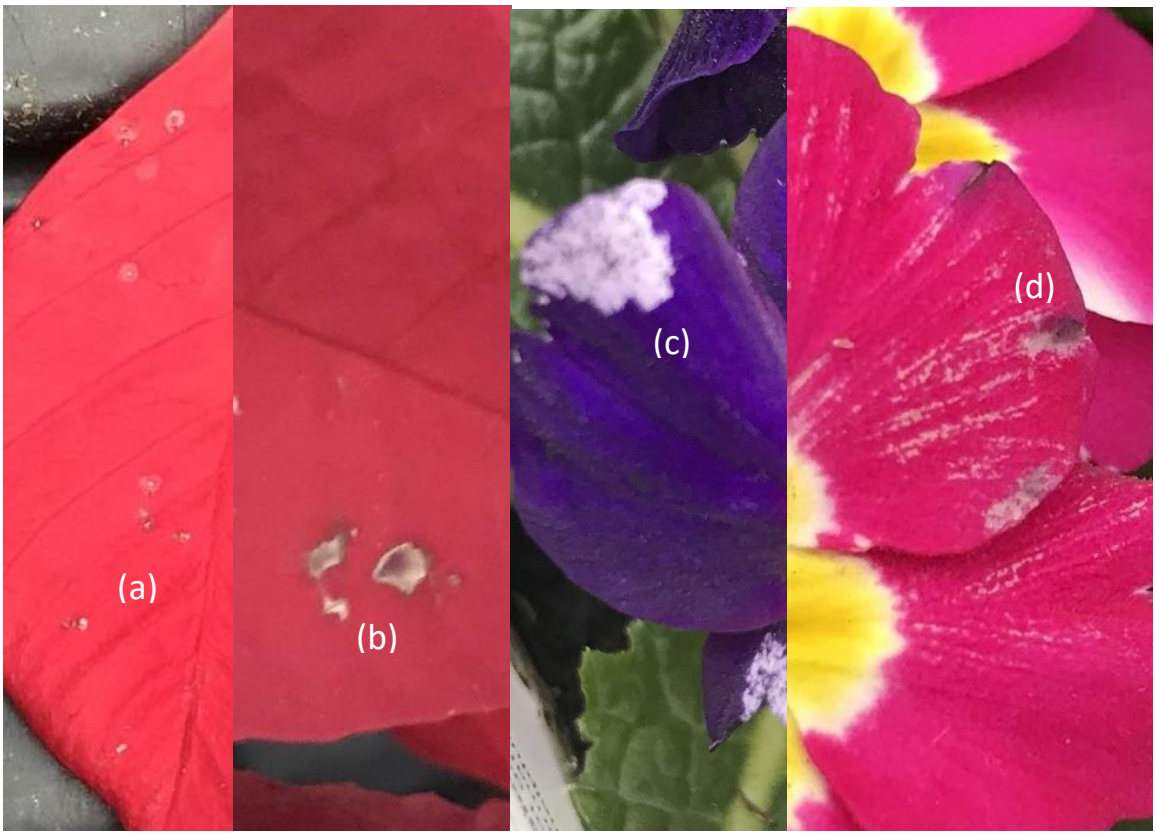

Fig. 3-12 Poinsettia bract and primula flower damage response after the combined application of *Botrytis cinerea* inoculation, UV-C exposure and storage during a shipping simulation. (a) *Botrytis cinerea* lesions on poinsettia bract of a control plant (b) *Botrytis cinerea* lesion on poinsettia bract with UV-C bleaching (c) UV-C bleaching on primula flower (d) *Botrytis cinerea* lesions on primula petals of control plants.

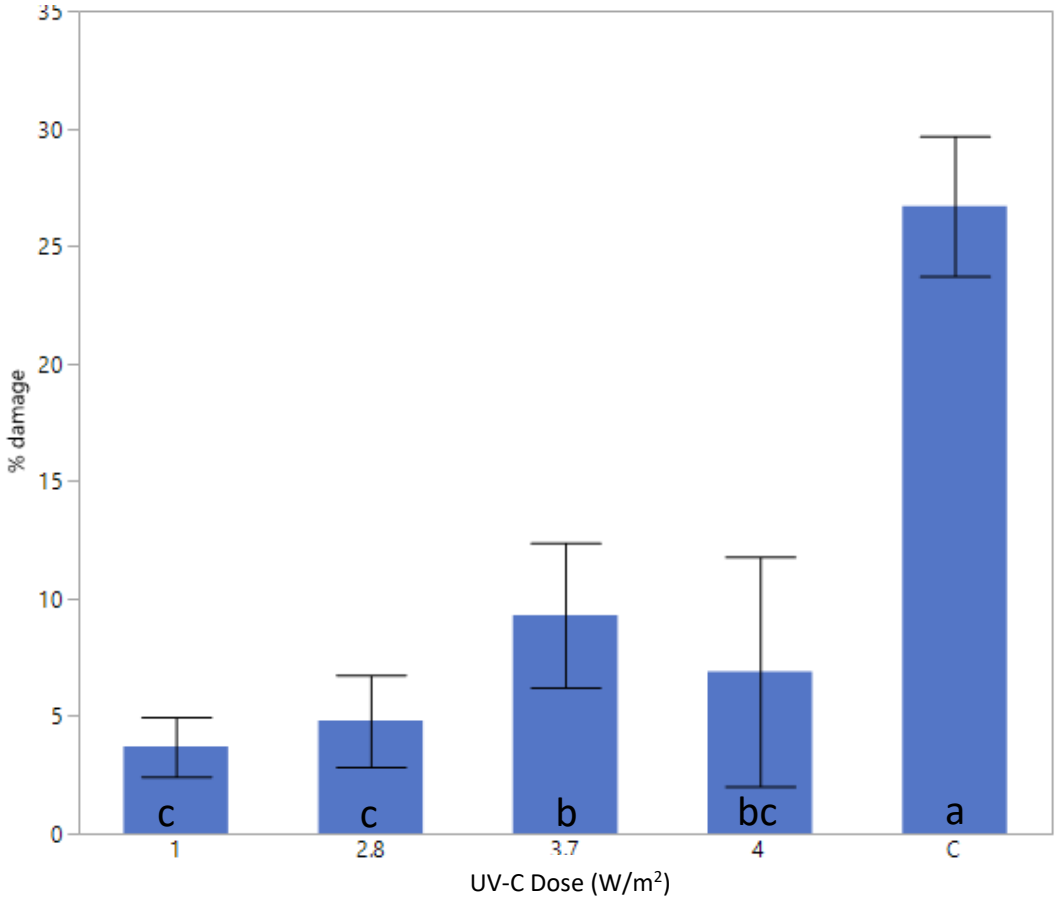

Fig. 3-13 Mean percent damage on poinsettia bracts 24 hours after shipping simulation, UV-C exposure and *Botrytis cinerea* inoculation. Error bar is constructed using a 95% confidence interval. Mean comparisons through Student's t-test and were constructed through a connecting letters report.

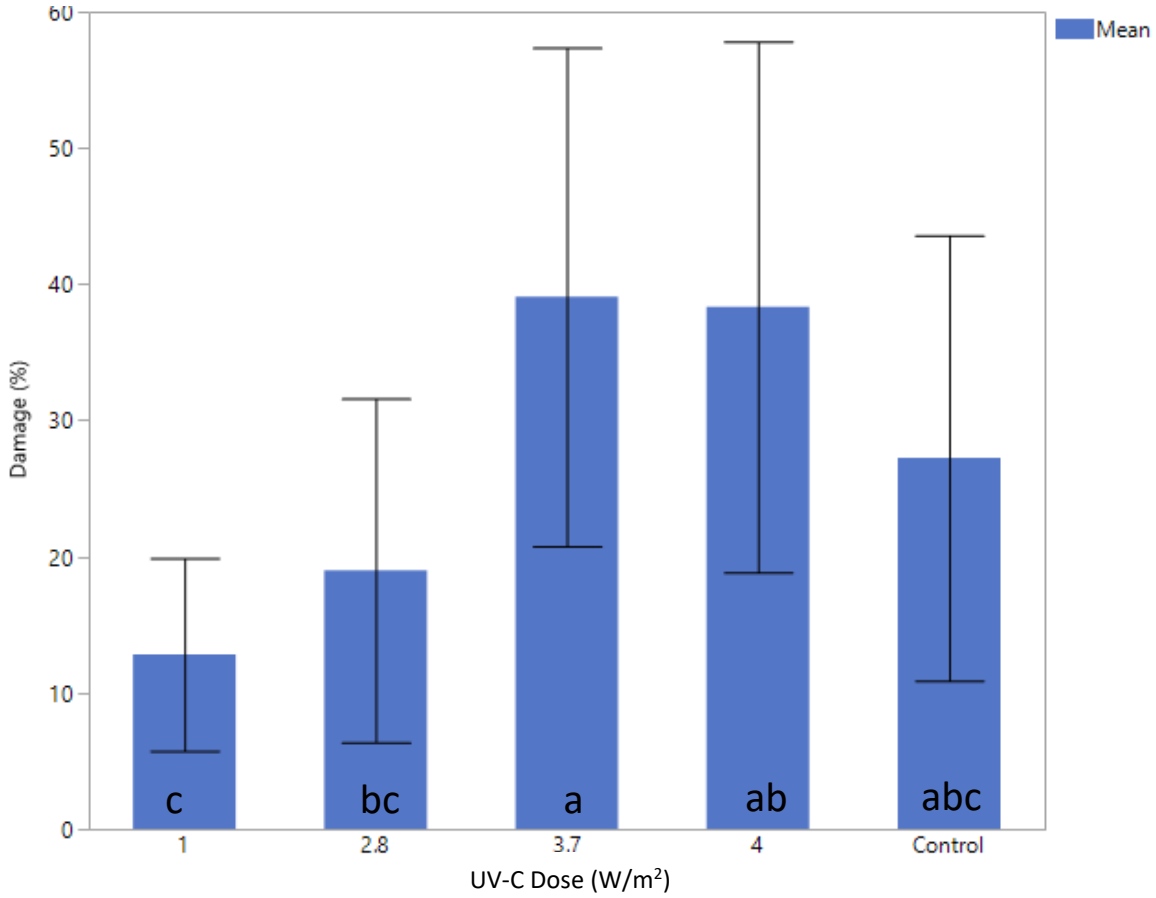

Fig. 3-14 Mean percent damage on primula flowers 24 hours after *Botrytis cinerea* inoculation, UV-C exposure, and shipping simulation. Mean comparisons using Student's t-test and were constructed through a connecting letters report.