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An Evaluation of Biopesticide Combinations on Yield Performance and Disease/Arthropod Control of Strawberries Grown in High Tunnel Plasticulture Production Systems in Arkansas.

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An Evaluation of Biopesticide Combinations on Yield Performance and Disease/Arthropod Control of Strawberries Grown in High Tunnel Plasticulture Production Systems in Arkansas.

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Horticulture

by

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This thesis is approved for recommendation to the Graduate Council.

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ABSTRACT

This two-year study investigated combinations of biopesticides to determine impacts on strawberry fruit marketable fruit yields, and effectiveness in controlling strawberry pests in a high tunnel production system at the University of Arkansas, Division of Agriculture Research and Extension Center in Fayetteville, Arkansas. Two strawberry cultivars *Fragaria × ananassa* (Duch.), Camino Real and Sweet Sensation were grown in a high tunnel from early-October to mid-May for two consecutive growing seasons, (2017-18 and 2018-19) with six treatment combinations of biopesticides including an untreated (water) control, nutrient spray and selected biological based fungicides and insecticides, arranged into a split-plot randomized block design. The cost associated with each biopesticide treatment combination was calculated based on the number of times applied to the specific area of the study and the cost of the products. Relative humidity, daily light integral (DLI) and growing degree days (GDD) were also recorded to show differences between the two growing seasons. During the 2018 season, the control (water) treatment numerically had the highest total and marketable fruit weight, but was not significantly different from any biopesticide treatment. No significant effects of biopesticide treatment were observed during the 2019 season on fruit yield or quality, thus indicating that there was no clear advantage to any of the treatments on improving fruit marketability. The biopesticide combination treatments were also evaluated for their impacts of four high tunnel pests of strawberry, powdery mildew (*Podosphaera aphanis* (Wallr.) U. Braun and S. Takam. (formerly *Sphaerotheca macularis* (Wall. Ex Fries) Jacz f. sp. *Fragariae* (Peries))), gray mold (*Botrytis cinerea*), two-spotted spider mites (*Tetranychus urticae* (Koch) (Acari: Tetranychidae)), and strawberry aphids (*Chaetosiphon fragaefolii* (Cockerell) (Homoptera: Aphididae)). Disease incidence for 2018 was less than 16% for powdery mildew and less than 25% for gray mold.

Powdery mildew in 2019 had less than 1% of disease incidence and gray mold had less than 2% disease incidence. In 2018, two-spotted spider mite populations were greater than the economic threshold of 5 mites per leaflet, but populations remained below the economic threshold in 2019. Strawberry aphid populations were not present in either harvest season. Overall findings point to the evaluated combinations of biopesticides not having a significant effect on fruit marketability or disease/arthropod control. These findings indicate that the tested combinations should not be used by producers to control pests or improve marketable yield of strawberries in high tunnels.

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LITERATURE REVIEW

An Evaluation of Biopesticide Combinations on Yield Performance and Disease/Arthropod Control of Strawberries Grown in High Tunnel Plasticulture Production Systems in Arkansas.

LITERATURE REVIEW

Strawberries

Wild *Fragaria* (Rosaceae) species occur naturally across the northern hemisphere and South America (Liston et al., 2014). The first record of domestication of wild *F. chiloensis* species occurred over 1,000 years ago by the Picunche and Mapuche people in Chile (Finn et al., 2013). In 18th century France, Antoine Nicolas Duchesne documented the hybridization of *F. virginiana* (North American species) and *F. chiloensis* (South American species) that developed one of the latest domesticated plants: *F. × ananassa* (Duch), which is an octoploid species (Liston et al., 2014; Petrasch et al., 2019). *Fragaria* species are found in Asia, Europe, North America, and South America (Husaini, 2016); two of these species: *F. moschata* and *F. vesca* were commercially cultivated for hundreds of years; however, production of these two species is uncommon due to the success of *F. × ananassa*.

All strawberry species are low growing, herbaceous perennials with branching crowns (Petrasch et al., 2019) and axillary buds which may form runners (stolons) for asexual reproduction or branching crowns (Hancock, 2000; Pritts et al., 1998). Strawberries are self-fertile, yet strawberry flowers are cross-pollinated by wind and insects, which is known to increase the size of fruit and increase yield (Johnson et al., 2014; Pritts et al., 1998). Additionally, *Fragaria* species have similar vegetative attributes with evergreen trifoliate leaves except for certain Chinese species having five leaflets (Liston et al., 2014), along with similar flower attributes that are actinomorphic (radially symmetrical) and differing species can have both perfect and imperfect flowers. Liston et al. (2014) goes on to detail that mature fruits are diverse between strawberry species, yet identification is based on physical attributes of the leaves and mature fruits. The mature fruits can vary within a species based on color, shape, achene and

calyx positions (Liston et al., 2014; Staudt, 1999). Variants within the species can occur from damaged and misshapen fruit due to disease incidence, arthropod damage, pollination problems and cold weather (Kandemir et al., 2019). Auxins are the primary growth regulator (synthesized in the achenes) that develops the receptacle tissue (Pritts et al., 1998). The strawberry's achenes are the botanical single fruits fixed to the fleshy receptacle similar to floral meristem tissue (Hollender et al., 2012), which classifies the strawberry as an aggregate accessory fruit, not a true berry (Darrow, 1966; Liston et al., 2014; Pritts et al., 1998).

Classifications of strawberry plants include short-day cultivars (also known as June-bearing or spring-bearing cultivars), day-neutral cultivars, and everbearing cultivars (Pritts et al., 1998; Samtani et al., 2019). Short-day cultivars begin flower initiation during days shorter than 14 hours; day-neutrals produce flowers and branch crowns during the season until temperatures reach 30°C; and everbearing cultivars produce flowers during the entire duration of the season except early spring when fruit is initiated (Durner et al., 1984; Pennsylvania State University, 2013; Samtani et al., 2019). Crowns are the growing point for strawberry plants, that produce leaves, stolons, branch crowns, and flowers (Pritts et al., 1998). Axillary buds are located at the axil (base) of a leaf and will grow shoots that can be either stolons or branch crowns dependent upon temperature and day length (Pritts et al. 1998). Historically, strawberries were grown within matted row systems which relies on the production of stolons (daughter plants) to maintain perennial production. However, perennial production is not the commercial standard for strawberry production in the U.S. The standard commercial production system is called annual hill plasticulture, which was developed in California (O'Dell and Williams, 2009; Samtani et al., 2019). This system decreases pest problems because there are new plants each year (Pritts et al., 1998). Annual hill plasticulture was introduced in the 1980's to the mid-southern states by NC

State University, annual plasticulture systems implemented methods of building raised beds over fumigated soil, covered with black plastic mulch with drip irrigation tape under the plastic for efficient watering (Poling, 2005; Samtani et al., 2019). It was a common practice to use methyl bromide as a fumigant for strawberry annual hill plasticulture production (Poling, 2005); however, this fumigant was restricted and phased out of use in 2005 due to the chemical's impact on depleting the ozone layer (EPA, 2018). It was observed by Poling (2005) that annual hill plasticulture is more productive than the perennial matted row system.

World strawberry production in 2018 was 372,361 hectares with China leading with 111,132 hectares followed by Poland (47,833 ha) and the U.S. (19,919) (FAO, 2020). The U.S. strawberry industry produced 1,296,272 tonnes of fruit following China (2,964,263 tonnes), but the FAO (2020) calculated yield for 2018 which placed the U.S. as having the most efficient production at 650,772 kg/ha. Within the U.S. California has the ideal climate for strawberry production with stable temperatures (Wortman et al., 2016), which makes California the largest producer of strawberries, in the U.S. is valued at \$2.3 billion USD, which makes up 87.64 percent of the market; the other states that make up the market value include (by percentage) Florida (10.55), North Carolina (0.80), Oregon (0.44), Washington (0.34), and New York (0.23) (USDA ERS, 2019). Other states with less than 500 acres of production are not listed within the USDA ERS statistics for the market value (E. Garica, personal communication). Total supply of U.S. strawberries in 2018 was 2,648.8 (million pounds) with 2,291.9 (million pounds) being utilized U.S. production and 356.9 (million pounds) imported. (USDA ERS, 2019). The 2018 U.S. strawberry industry market was valued at \$2.67 billion USD with 9% being processed and 91% being for the fresh market with 7.14 pounds per capita use, which greatly increased from 1.97 pounds per capita use in 1980 (USDA ERS, 2019). Arkansas strawberry production is

estimated to be only 63 acres of total production (Samtani et al., 2019). Possible limitations to production in Arkansas include problems associated with clay heavy soil, weather conditions and high pest pressure. Growers in Arkansas and other small production states (particularly within the Mid-south and Southeast) usually sell to direct market through by farmer's markets and u-pick operations (Poling, 2005; Samtani et al., 2019).

Rysin et al., (2015) found that annual production costs for conventional plasticulture systems had an estimated value at \$18,621 USD per acre and estimated costs for organic systems were valued at \$23,376 USD per acre. The conventional system profited with an estimated gross \$33,600 USD per acre producing 1.02 pounds of fruit per plant and the organic system profited with an estimated gross \$42,770 USD per acre producing 0.94 pounds of fruit per plant (Rysin et al., 2015). Strawberry production has relatively high input costs, so to make a profit growers need to be able to produce 1-1.2 pounds of fruit per plant (Poling, 2005). For U.S. producers that sell to the retail market and not to direct markets such as farmer's markets or u-pick operations, the retail value of strawberries sold in 2018 was \$2.88 USD per pound (USDA ERS, 2019). Retail values for strawberry fluctuate with lower average prices per pound during the summer and higher averages in winter months. The USDA ERS (2019) tracks prices received by growers, and for early production in March 2019 the price received was \$114 US per hundredweight (cwt) and when the market was saturated by April 2019 the price received dropped to \$79.20 US per cwt. Arkansas' strawberry market is based in farmer's markets and u-pick operations between mid-April to early June (Samtani et al., 2019), so if growers are able to produce fruit earlier the price received would increase especially for locally produced fruit.

High Tunnels

An option for Arkansas growers to produce strawberries for earlier markets, control rain and mediate temperature is through the use of high tunnels. A high tunnel is a structure that is similar to a greenhouse and primarily provides protection from weather. High tunnels offer a semi-controlled environment by protecting plants from rain and temperature fluctuations (Janke et al., 2017). These structures are traditionally built using curved metal arches covered with UV treated polyethylene plastic and utilize side and end walls that are manually rolled up/down for passive ventilation (Janke et al., 2017); high tunnels come in many sizes as single or multi-bay structures and styles as Quonset (hoop) or gothic (arched) (Carey et al., 2009; Janke et al., 2017). Unlike greenhouse systems, in high tunnels plants are typically grown in the soil (Pottorff and Panter, 2009) and there are little to no automatic temperature control systems (Bruce et al., 2019; Carey et al., 2009; Lamont, 2009). Heat from the sun is captured inside the structure by the use of UV treated polyethylene plastic which diffuses light and holds heat inside the tunnel. The tunnel environment protects plants from wind and cold damage in winter months, and growers can also implement the use of row cover or small heaters for added protection during the coldest months (Janke et al., 2017).

High tunnels are increasing in popularity for specialty crop production globally and in the United States (U.S.) (Lamont, 2009). China has the most hectares of high tunnel construction worldwide, whereas the U.S. has only a small portion (XX) most of which are concentrated in California, Florida, New York and Pennsylvania (Bruce et al., 2019; Janke et al., 2017; Lamont, 2009). The USDA Natural Resource Conservation Service (NRCS) initiated a cost-share program that aids growers to purchase high tunnels with the goal of extending the growing season of certain crops such as vegetables and small fruits, which has increased the popularity of

high tunnels in the U.S. (USDA NRCS, 2018). Even with programs that provide cost savings, Janke et al., (2017) concluded that the initial cost for building a high tunnel (about \$10,000 for a single bay and \$40,000 for a multi-bay) may not be offset if crop failure occurs or if high tunnel production is not as profitable as a crop grown in a field system.

Benefits of high tunnels includes the potential to increase yield and crop quality by reducing precipitation that can promote fungal infections, decreasing arthropod activity, and extending the production season (Ingwell et al., 2017) by manipulating temperature and light (Grijalba, 2015; Verheul et al., 2006; Wortman et al., 2016). From the Midwest to Canada high tunnels have been shown to result in a season that can be up to five weeks earlier in the spring and up to a month longer into the fall than field production (Janke et al., 2017; Kadir et al., 2006). To maximize growing potential, Janke et al., (2017) concluded that high tunnels need to be oriented toward specific cardinal directions based upon the grower's latitudinal location, and since Arkansas is below 40° latitude then a high tunnel is best oriented north to south to maximize light intensity. Another possible benefit of high tunnels is the added protection from pests common in field production; however, some researchers have concluded that high tunnels do not deter arthropod activity, and instead may provide a protected, ideal environment for arthropods such as aphids and mites to thrive (Johnson et al., 2010; Ingwell et al., 2017).

A possible negative attribute of high tunnels is the recurring costs to replace the polyethylene plastic every few years due to age or if hail and wind damaged occurs (Janke et al., 2017) High tunnel production also creates additional labor demands which can potentially offset increased profits (Waldman et al., 2012); labor is needed to manually open/close the tunnel to manage temperature plus extended harvest periods require added labor cost. High tunnels have

the capability to produce crops at any time, so growers can carefully plan certain crops for year round production (Bruce et al., 2019; Waldman et al., 2012).

The most popular crops produced in high tunnel systems are vegetables; however, there has been an increased popularity in berry production in the United States and Canada (Demchak, 2009). Researchers in Florida found that early yields of strawberries increased approximately 54% inside of the tunnel and that fruit weight was approximately 63% higher when comparing the high tunnel system to the open-field system (Salame-Donoso et al., 2010). It is reported that yield and profits are increased on strawberries grown in protected environments due to higher fruit quality and earlier harvest periods (Kandemir et al., 2019).

The use of high tunnels can help mitigate Arkansas' environmental conditions such as rainfall, temperature fluctuations and light conditions that make crop production, specifically strawberry production, difficult in the state. The demand for locally grown food is encouraging growers to look toward options such as high tunnel production to make crop production more profitable in less than ideal environmental regions (Rowley et al., 2011).

Strawberry High Tunnel Pests

Powdery Mildew

Powdery mildew or *Podosphaera aphanis* (Wallr.) U. Braun and S. Takam. (formerly *Sphaerotheca macularis* (Wall. Ex Fries) Jacz f. sp. *Fragariae* (Peries)) is a common disease associated with yield loss and poor fruit quality from infection of leaves and fruit tissues (Maas, 1998). High tunnels have greater powdery mildew incidence because of increased relative humidity creating a favorable microclimate for the disease (Demchak, 2009; Xiao et al., 2001). Powdery mildew conidia are dispersed by wind, which is minimized by using high tunnels (Blanco et al., 2004). Symptoms include white, mycelial growth that infects plant foliar tissues

and fruit, which can cause malformation or abortion (Peres and Mertely, 2009). Disease-free plugs and fungicides are the best management practices for powdery mildew control (Maas, 1998). The use of fungicides for control of powdery mildew in strawberry can be of concern due to pre-harvest intervals and potential for resistance development for some areas. Carisse et al., (2013) created three statistical models to predict powdery mildew incidence-severity relationships for June-bearing cultivars in open field and high tunnel systems and open field day-neutral cultivars by counting diseased leaves to improve knowledge of the disease's epidemiology to make decisions regarding fungicide application. It was concluded that the high tunnel had a higher incidence-severity than the open-field conditions, which shows that high tunnels have a more favorable environment for powdery mildew incidence-severity than the open-field (Carisse et al., 2013). There has been successful breeding for powdery mildew resistance strawberry plants using wild-type genomes in Florida (Kennedy et al., 2013). Interestingly, powdery mildew is often associated with subsequent infestations of two-spotted spider mites, *Tetranychus urticae* (Koch) (Asalf et al., 2012).

Gray Mold

Gray mold, also known as *Botrytis cinerea*, is considered to be the fungal pathogen that causes the greatest economic damage to the strawberry industry (Petrasch, et al., 2019). Gray mold can cause crop losses up to 15% in Florida (Legard and Chandler, 1998; Xiao et al., 2001) and wet conditions can result in even greater losses during fruit set (about 80%) without the use of fungicides (Ries, 1995). Control of gray mold is difficult because it has a wide host range of more than 200 crop species globally (Williamson et al., 2007), but recent research from Elad et al., (2016) indicates that number to have increased to over 1,000 plant species that can be infected by gray mold. This is indicative to the fact that gray mold has multiple methods of

infecting plant by direct contact, airborne conidia or infected flowers hosts through different inoculum sources (Williamson et al., 2007).

Gray mold is a necrotrophic disease that infects damaged leaves or fruit (Petrasch, et al., 2019). Conidia will grow from infected tissue (Jarvis, 1962) and then disperses into natural openings or damaged tissues of nearby plants (Holz et al., 2007). Gray mold can infect strawberries in two ways: primary infection occurs in open flowers and secondary infection occurs in the fruit receptacle tissue (Bristow et al., 1986). Primary infection is initiated by conidia from adjacent infected plants (Jarvis, 1962) and then goes into an asymptomatic or quiescent phase until fruit ripens and then tissue is quickly destroyed (Williamson et al., 2007). Mechanisms of the asymptomatic or quiescent phase is not fully understood at this time (Petrasch et al., 2019). Secondary infection does not have an asymptomatic phase, which causes immediate decay and is initiated by direct contact from infected leaves and fruit (Holz et al., 2007; Jarvis, 1962). Strawberries are fairly resistant to gray mold until the ripening stage occurs when the cell walls and cuticle change and sugars begin to accumulate within the fruit (Petrasch et al., 2019).

Methods of control include combining efforts of sanitation practices and fungicide application to reduce the severity of gray mold incidence. Sanitation begins with removal of infected fruit and foliage along with senescing flowers in the beginning of fruit development (Daugaard, 1999). Several practices such as not allowing fruit to make contact with soil by using plastic mulch to cover the strawberry beds (Daugaard, 1999), creating an open canopy (Williamson et al., 2007) or using drip irrigation to keep plants dry so to reduce inoculum spread (Dara et al., 2016; Terry et al., 2007). The use of high tunnels can help greatly with reducing the spread of air-borne spores to strawberries (Xiao et al., 2001). Wedge et al. (2007) recommends

using a variety of fungicides with different modes of action to decrease resistance. For organic production, most fungicides used for gray mold are *Bacillus* based (Pertot et al., 2017), but high costs limit the commercial use of biological control products (Petrasch et al., 2019). Prokkola and Kivijarvi (2007) reported that losses on organic or unsprayed strawberry trails ranged between 3.7%-27.5% from gray mold incidence. To date there are no fully resistant strawberry cultivars to gray mold (Bestfleish et al., 2015; Bristow et al., 1986). Certain cultivars can have less severity compared to others, but all fruit can be infected pre- and post-harvest (Lewers et al., 2012). In the efforts of breeding a resistant cultivar to gray mold, there is a theory that some wild strawberries could parent a genetically resistant cultivar or at least decrease incidence severity; however, this has yet to be proven (Petrasch et al., 2019).

Two-Spotted Spider Mites

Two-spotted spider mites, *Tetranychus urticae* (Koch) (Acari: Tetranychidae) is a serious arthropod that causes severe damage to strawberries and other crop hosts throughout temperate and subtropical regions (Fasulo and Denmark, 2000). Two-spotted spider mites feed on the underside of leaves by sucking sap which gives a bronzed, mottled appearance and severely infested plants are covered by webbing with reduced yields from depleted leaf nutrients (Bessin, 2019; Fasulo and Denmark, 2000; Howell and Daugovish, 2013). Reduced yields are not from lower weight, but from reduced number of fruit (Walsh et al., 1998). Low soil moisture, high temperatures, and dusty conditions promote two-spotted spider mite populations with almost three times as many eggs and motiles on strawberry plants (Godfrey, 2011; White and Liburd, 2005). Optimal temperature for TSSM population development is around 30°C, which is easily reached within high tunnel systems (Bounfour and Tanigoshi, 2001; Fasulo and Denmark, 2000; Park and Lee, 2005). Adults are almost microscopic with males measuring 1/80 inch (0.3mm)

and females measuring at 1/60 inch (0.4 mm) which can lay between several hundred eggs in their lifetime (Bessin, 2019; Fasulo and Denmark, 2000). TSSM life stages begin with the egg, then larval stage, protonymph and deutonymph stages, and then finally the adult stage, which can be as short as five days to as long as twenty days depending on conditions (Fasulo and Denmark, 2000). Insecticide use can actually deplete numbers of beneficial insects so the recommendation is to use pesticides sparingly. Natural enemies that effectively control two-spotted spider mite populations include *Phytoseiulus persimilis* (Athias-Henriot) (White and Liburd, 2005), *Neoseiulus californicus* (McGregor), *N. fallacis* (Garman), and *Amblyseius andersoni* (Chant) (Howell and Daugovich, 2013). Economic threshold of two-spotted spider mites is five mites per leaflet (Burrack, 2017) and injury threshold is measured in cumulative mite days (CMD) per leaflet (Hull and Beers, 1990).

Biological Control

Organically produced and marketed products have become readily available to U.S. consumers due to increasing demand from the early 2000's. The Organic Trade Association (OTA) marks millennials as the driving force for more organic production with demands of transparency and integrity from the market (2019). Other influencing factors are presented through organizations such as the Environmental Working Group (EWG), which claims certain food crops are laden with pesticide residues. The EWG provides a list called the Dirty Dozen®, which ranks food crops with high pesticide residues. However, the EWG gives biased evaluations and their methods have been debunked in published literature. Strawberries are listed as the number one crop having the most pesticide residues found within the tested group on the Dirty Dozen® (EWG, 2019).

By 2005, organic fruit and nut management was 2.5 percent of the organic market and organic food sales reached \$21.1 billion USD in 2008 (Greene et al., 2009). A decade later, the OTA (2019) reported that organic food sales more than doubled at \$47.9 billion USD in 2018. Even with the increase in demand and sales in the organic market, a producer's decision to adopt organic practices is largely influenced by perceived risks such as cost, yield loss to diseases, pests, soil fertility, weather and weeds; furthermore, changes in climate with fluctuating weather increase the potential risk of pressure from diseases, pests and weeds (Mader et al., 2002; Veldstra et al., 2014). To produce organically means to follow a set of guidelines for production standards set by the USDA National Organic program which only includes natural or organically labeled products such as pesticides and fertilizers (McWhirt et al., 2014). Organic production can produce lower yields during the first few years of production (Azadi et al., 2011; Mader et al., 2002), yet production can increase with long-term organic practices because it addresses problems in a sustainable manner such as using legumes for nitrogen fixation rather than using synthetic nitrogen fertilizers (Badgly and Perfetto, 2007).

Mounting pressure on the agricultural industry to produce more sustainable crops on less land is already an obstacle in food production and security. Unlike organic production, sustainable production or practices do not have to follow a specific set of guidelines or standards (McWhirt et al., 2014). Sustainability is not easily achieved when arthropods, diseases, weeds and other crop pests cause an estimated 40% loss in crop production (Chandler et al., 2011; Glare et al., 2012). For producers who wish to use any organic products must be approved and certified by the Organic Materials Review Institute (OMRI) and applied from sprayers utilized for organic products only, which can increase the cost of production and input costs. Options available for

organic producers include purchasing disease free plants, monitoring and scouting, and using OMRI approved pesticides including biopesticides.

The United States Environmental Protection Agency (EPA) (2018) defines biopesticides as types of pesticides produced from natural materials: animal, plant, bacteria and certain minerals. Another definition is that biopesticides are a mass-produced product derived from living organisms or natural products to be sold as plant pest controls (Chandler et al., 2011). While biopesticides are considered a new or niche technology in today's agriculture industry, the EPA had established the Biopesticides and Pollution Prevention Division in the Office of Pesticide Programs in 1994 (EPA, 2018). The EPA requires less data to register a biopesticide so the process can take less than a year for a biopesticide rather than a conventional pesticide, which takes three or more years to become registered. As of 2016, the EPA has 299 registered biopesticide active ingredients and there are 1,400 biopesticide product registrations.

An increase in strict pesticide regulations and consumer demand are the driving factors for Europe to become the fastest growing market to adopt biopesticides (Chandler et al., 2011). North America is the leading continent that uses biopesticides making up 45% of the market, then follows Europe with 20% of the biopesticide market, Oceania 20%, South and Latin America 10%, and Asia 5% (Bailey et al., 2010; Copping, 2014). An increase in value and demand for natural products in the United States has developed a need to expand research and market production of biopesticides even while these products have been used for over 100 years (Arthurs, 2018). Even with the growth in market value, biopesticides are still slowly being adopted by growers due to high costs of the product, lack of efficacy, inconsistent field trials, great expectations of the product, quality control issues, short shelf-life, and lack of awareness that are associated with the biopesticides as a niche market (Arthurs, 2018; Chandler et al., 2011;

and Glare et al., 2012). Biopesticides make up a small portion (approximately 5%) of the pesticide market, the estimated value for 2017 was \$3.2 billion USD (Copping, 2014; Damalas et al. 2018). The expected growth of the market from 2017 to 2022 is 15.43% CAGR, approximately 6.60 billion USD (Markets and Markets, 2018); exceeding the 3% expected for conventional pesticides (Arthurs, 2018).

Biopesticides are subcategorized as: (1) microbial, (2) biochemical, (3) semiochemicals, and (4) plant-incorporated protectants (Chandler et al., 2011; EPA, 2018). Microbial pesticides are composed of bacteria, fungi, oomycetes, viruses and protozoa, and are emerging as the more popular category of biopesticides (Arthurs and Dara, 2018, Chandler et al., 2011 and Dunham, 2015). Naturally produced pyrethrins and neem oil are two of the most used biochemical based biopesticide products on the market (Chandler et al., 2011). According to Chandler et al (2011) biochemical products have a low toxicity rate toward mammals and degrade quickly after application; however, some resistance has developed since the introduction of biochemicals in 1997 with western flower thrips and tetranychid mites. Semiochemicals, such as insect pheromones, are used to cause a behavioral change in same or different species of the specified pest (Chandler et al., 2011); however, the EPA (2018) does not classify semiochemicals as a biopesticide category. A large aspect of certified organic production is the use of pesticides not synthetically developed for control of disease and arthropods, with the exception of plant-incorporated protectants (PIPs), commonly used in the U.S. (Marrone, 2014). PIPs are pesticide substances added to a plant that are produced from other genetic material, such as genetically modified crops (EPA, 2018). Europe does not classify PIPs as a biopesticide category due to consumer resistance to genetically modified (GM) crops (Chandler et al., 2011).

The main benefit of using biopesticides is that they are usually less toxic to the environment than conventional pesticides and have less risk to humans (Damalas et al. 2018; Hubbard et al., 2014). The EPA has determined other benefits of biopesticides include targeting specific pests rather than being broad spectrum and that they are effective in small quantities and decompose quickly leading to lower exposure rates and pollution problems (EPA, 2018). By integrating pest management protocols, the use of biopesticides will decrease pest problems and increase crop yields (Chandler et al., 2011 and EPA, 2018).

Environmental Conditions

Climatic conditions can make a substantial impact on each growing season. Weather is an ever-changing phenomenon with certain events such as rain, freezing temperatures or drought causing crop damage. Light and temperature are the driving factors for plant, disease and arthropod growth and development. Light and temperature are more accurately measured as daily light integral (DLI, $\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$) for the amount of light photons gained (Runkle E. 2006) and growing degree days (GDD) to accurately distinguish weather and climate effects (Skaggs et al. 2012). DLI is an essential measurement of photosynthetically active radiation (PAR) ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), which is a spectral range from 400-700 nm, that measures light intensity in certain locations for each day received (Runkle E. 2006; Torres et al., 2012). Runkle E. (2006) stated that DLI is measured by number of moles (mol) per meter squared (m^{-2}) per day (d^{-1}) and the optimal amount of DLI is between 10-12 $\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ for greenhouses. Each crop has a different base temperature where growth and development occurs, for strawberries this point occurs at 10°C (50°F). This base temperature (10°C) along with the average of the maximum and minimum temperature for a given day indicates the amount of time heat accumulates called growing degree days at base 10 (GDD_{10}). For strawberries grown in Arkansas, GDD_{10} begins

accumulating at the beginning of planting in late September / early October and ends at the time the plants are terminated at the end of harvest season in May. This time-frame will sum the GDD to find the cumulative GDD-10 for that season (Su et al. 2013). O'Connell et al. (2012) found that a high tunnel is capable of increasing temperature and GDD₁₀ due to the plastic covering the structure. High tunnels can promote more optimal temperatures for crop production than the open field; Hunter et al. (2012) found that during the day high tunnels can be up to 10 degrees or higher than outside temperatures, yet only 1-4 degrees higher during the night.

OBJECTIVES

To determine the effect of commercially available biopesticide combinations on yield performance of two strawberry cultivars: Camino Real and Sweet Sensation, grown in plasticulture production systems in high tunnels, (2) to assess environmental conditions during the growing season, (3) to provide a cost comparison for the biopesticide treatments, and (4) to test the efficacy of these biopesticide combinations in controlling three strawberry pests.

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CHAPTER 1:

Effect of commercially available biopesticide combinations on yield performance of two strawberry cultivars: Camino Real and Sweet Sensation grown in high tunnel plasticulture production systems while assessing environmental conditions and providing a cost comparison for the biopesticide treatments

ABSTRACT

Options for organically grown strawberries in Arkansas are limited due to problems with weather/climate conditions and disease/arthropod pressure. This study was conducted in 2018 and 2019 to determine the effect of six combinations of biopesticides on fruit marketability for two strawberry cultivars: Camino Real and Sweet Sensation grown under high tunnel. The cost associated with each biopesticide combination was evaluated for the number of times applied to the specific area of the study. Relative humidity, daily light integral (DLI) and growing degree days (GDD) were also recorded to show differences between the two growing seasons. The biopesticide combination treatments significantly affected total, and marketable strawberry fruit weight during the 2018 season where the control (water) treatment had the highest total and marketable fruit weight (1,950.62 g and 1,042.44 g, respectively). However, there was no treatment effect during the 2019 season. Based on these results, none of the biopesticide combinations had a clear advantage for improving fruit number or weight. Yields were relatively low for both seasons at 227 g of fruit per plant. Each treatment area totaled 280m² and the combination treatment costs were evaluated for one application and five applications in a season. For one treatment application, all of the biopesticide combinations were under \$3.50 USD and for five treatment applications the cost per biopesticide combination was under \$17 USD. Labor, equipment, and other inputs were not included within the cost. DLI in 2018 was within the normal DLI hours for Arkansas, but 2019, it was lower than the normal range. GDD was also lower in 2019 for both the field and high tunnel than 2018. Relative humidity for both seasons were at similar levels.

INTRODUCTION

The U.S strawberry (*Fragaria × ananassa* Duch. industry is valued at \$2.67 billion USD (USDA ERS, 2019). Arkansas strawberry production is limited with approximately 63 acres of total production (Samtani et al., 2019), Arkansas growers and other small production states (particularly within the mid-south and southeast) sell direct market through farmer's markets and u-pick operations (Poling, 2005; Samtani et al., 2019). Farmer's markets and u-pick operations have gained popularity with consumers demanding locally grown food. However, problems such as the heavy clay soil, weather conditions and disease/arthropod pressure make locally grown strawberries difficult for growers.

Arkansas climatic conditions make strawberry production (and specifically organic strawberry production) difficult. Arkansas is prone to fluctuating temperatures and receives an average of 45 in (1,143 mm) of rain per year (National Weather Service, 2020). Stable weather and climatic conditions are very important for strawberries, California has the ideal climate for strawberry production (Wortman et al., 2016) and it produces 88% of U.S. strawberries valued at \$2.3 billion USD (USDA ERS, 2019). Light and temperature are the two most important factors for plant growth and development. Light and temperature are more accurately measured as daily light integral (DLI) for the amount of light photons gained (Runkle, 2006) and growing degree days (GDD) for accumulated heat units to accurately distinguish weather and climate effects (Skaggs et al. 2012). According to Faust and Logan (2018), the normal DLI range for Arkansas in February is 20-25, March is 25-35, April is 35-40, and May is 40-45. GDD begins at a base temperature, which is 10°C for strawberries. GDD-10 begins accumulating at the beginning of planting and ends at the time the plants are terminated at the end of harvest season in May. This time-frame will sum the GDD to find the cumulative GDD-10 for that season (Su et al. 2013).

High tunnels (HT's) can help provide producers with the solution to mitigate the weather problems Arkansas growers face. High tunnels are structures covered with UV treated polyethylene plastic that has side and end walls to allow passive ventilation (hightunnels.org; Janke et al., 2017; Bruce et al., 2019). Benefits to using a high tunnel include protection from rain and temperature fluctuations, potential to increase yield, decrease disease infections and arthropod infestations, and extend the production period by diffusing light and manipulating temperature (Grijalba, 2015; Ingwell et al., 2017; Janke et al., 2017; Verheul et al., 2006; Wortman et al., 2016). These benefits increase the potential sustainable and profitable production of strawberries in less than ideal environmental regions such as Arkansas (Rowley et al., 2011).

Arkansas conditions also create a favorable environmental conditions for disease and arthropod pressure. One general benefit to the use of high tunnels is the decreased disease and arthropod incidence; however, in some instances, high tunnels can instead provide a favorable environment to pests that would not be an issue in the field (Jordan and Hunter, 1972; Mass, 1998; Xiao et al., 2001). Grower's options for control include purchasing disease free plants, sanitation practices, and chemical control. Consumer demand is moving toward more organic and sustainable options when it comes to chemical controls. Sustainability is not easily achieved when arthropods, diseases, weeds and other crop pests cause an estimated 40% loss in crop production (Chandler et al., 2011; Glare et al., 2012). Input costs for strawberry production are relatively high and producers should expect an estimated 0.5 kg of marketable fruit per plant for a break-even point (Poling, 2005). Rysin et al., (2015) found that annual production costs for conventional systems had an estimated value at \$18,621 USD per acre, which profited an estimated gross \$33,600 USD (net \$14,979 USD) and estimated costs for organic systems were valued at \$23,376 USD per acre, which profited as estimated gross \$42,770 USD (net \$19,394).

Crop losses from disease and arthropod damage can cause significant problems for growers. An option for growers to satisfy consumer demand for organic products while also having some control over disease incidence and arthropod infestation is to incorporate biopesticides for pest control. Considered to be a small, niche market, biopesticides are expected to continue increasing within the pesticide market (Markets and Markets, 2019; Arthurs, 2018). North America currently has the largest use for biopesticides at 45% (Bailey et al., 2010; Copping, 2014). Biopesticides are naturally occurring compounds used for the control and elimination of pests (EPA, 2018). These compounds are sub-divided into four categories: microbial, biochemical, semiochemical, and plant-incorporated products (PIP's) (Chandler et al., 2011; EPA, 2018). Biopesticides are target specific to pests and cause less toxic problems to both the environment and humans (Damalas et al. 2018; EPA, 2018; Hubbard et al., 2014).

The objective of this study was to determine if selected commercially available biopesticide products had a significant effect on yield performance of two strawberry cultivars: Camino Real and Sweet Sensation, (2) to determine if the use of these products was cost effective, and (3) to assess environmental conditions within the tunnel.

MATERIALS AND METHODS

Site Location

This study was conducted at the University of Arkansas Agriculture Research and Extension Center (UAREC) in Fayetteville, AR (Latitude: 36.1N; Longitude: 94.1W; USDA Cold Hardiness Zone 6b; AHS Heat Zone 7), during the 2018 and 2019 harvest seasons. Strawberries were grown in an on-site, single bay ClearSpan™ Quonset-style high tunnel (FarmTek, Dyersville, Iowa) over Captina silt loam soil with a pH between 6.1 and 6.2 (Appx A). The tunnel was originally three separate tunnels but were put together to create a longer

tunnel that is 6 m by 41.5 m and oriented East to West. The tunnel was covered with a single layer, 6 mil UV treated polyethylene plastic with rolling down sidewall curtains and opening roll-up endwall doors for passive ventilation.

Production Management

All practices in this study were conducted according to the standards the Strawberry Production Guide for the Northeast, Midwest, and Eastern Canada (Pritts et al., 1998). Prior to planting in the high tunnel, Bermuda grass (*Cynodon dactylon*) had grown in the space and was tilled during the summer of 2017 and 2018. In the summer of 2018, a cover crop of assorted cow peas was planted to deter the re-establishment of Bermuda grass. Irrigation was applied using sprinklers to have the ground ready for building beds. Three beds were constructed within the tunnel to be approximately 91 cm wide by 39.6 m long and 1.2 m apart. One mil black plastic mulch that was 1 m wide from Harris Seeds (Rochester, New York) and five mil t-tape was applied under the plastic mulch for irrigation (T-Tape Drip Tape, John Deere, Moline, Illinois). Landscape fabric was stapled between raised beds to deter weed establishment (Samtani et al., 2019). The plants were ordered from McNitt Growers (Carbondale, Illinois) in the summer of 2017 and 2018 for the 2018 and 2019 harvest period. The strawberry plugs were delivered by the last week of September and planting occurred during the first week of October for both years. For winter protection, low tunnels were constructed over the beds using cut rebar and thin poly tubing with baling string to keep the row cover floating above the plants. The row cover was a two mil white fabric, custom cut from BWI Industries (Texarkana, Arkansas). The tunnel was closed if the lowest predicted temperature was below 7°C and the row cover was applied if temperatures reached 1.5°C or lower. Soil analysis was conducted by the UA Agriculture Diagnostic Laboratory (UAADL) for both harvest seasons (Appx. A). The high tunnel was

fertilized once during March 2018 with a Phosphorus focused fertilizer injected into the irrigation system using a Dosatron® D25RE2 (QC Supply, Lincoln, Arkansas) because soil analysis confirmed there was a phosphorus deficiency. Trifoliolate and petiole samples were taken in 2019, which indicated that all macro and micronutrients were at sufficient levels (Appx. B). Samples were only taken in 2019 due to lack of funding to conduct the analysis in 2018. This study was treated as annual plasticulture production so the plants were removed from the ground at the end of harvest and the same protocol was used for crop establishment the following year.

Cultivars

Two strawberry cultivars: Camino Real and Sweet Sensation were selected for this project. Camino Real was bred by Douglas V. Shaw and Kirk D. Larson at the University of California (Patent: USPP13079P2, Google Patents). It was patented in 2002 and is considered a short-day strawberry cultivar with good flavor and low unmarketable yields. ‘Camino Real’ was chosen because it is considered a widely used cultivar (A. McWhirt, personal communication). The cultivar, Sweet Sensation ‘Florida127’ was bred by Vance Whitaker at the University of Florida (Patent: USPP25574, University of Florida). It was released in 2013 and is comparable to two industry standards: ‘Florida Radiance’ and ‘Strawberry Festival’. ‘Sweet Sensation’ was chosen based on the recommendation that this cultivar is becoming increasingly popular within the organic strawberry community (B. McNitt, personal communication).

Biopesticide Combination Treatments

This study was conducted in conjunction with a Texas A&M University project titled, “Evaluating organic pest control products for strawberries in combination with high and low tunnels for limited resource farmers in the Mid-South” funded by a Southern SARE R&E grant (LS16-275). As a collective group, the collaborators decided on which commercially available

products would be tested within these studies. Several biopesticide products classified as fungicides (F) and insecticides (I) were selected then arranged into six treatment combinations (Appx. C).

1. Treatment “Control” = foliar application of water.

Water was used as the control treatment because of a previous study conducted at the UA Fruit Research Station where data was skewed due to plants not receiving the same degree of wetness during pesticide application, causing less disease on unsprayed plants (T.Ernst, personal communication).

2. Treatment “APA” = Actinovate SP® (F) + PyGanic® (I) + Actinovate SP® (F)

Actinovate SP® (Novozymes, Franklinton, North Carolina) is labeled as both a root drench and foliar spray for preventative suppression/control of powdery mildew and gray mold on strawberries. PyGanic 1.4® (MKG, Minneapolis, Minnesota) is a commonly used insecticide for the control of two-spotted spider mites and strawberry aphids.

Actinovate SP® was applied as a root drench at planting and then mixed with PyGanic 1.4®, a pyrethrin that controls aphids and mites, as a foliar spray during the 2018 and 2019 harvest seasons.

3. Treatment “ACM” = AmyProtec 42 (F) + Captiva® (I) + MilStop® (F)

AmyProtec 42 (Andermatt Biocontrol, Grossdietwil, Switzerland) is not yet commercially available to American growers and is labeled as a soil or root drench only. MilStop® (BioWorks, Victor, New York) is specifically labeled for powdery mildew on strawberries in the field and greenhouses. Captiva® (Gowan, Yuma, Arizona) is a registered insecticide for the control of mites used in combination as a foliar spray with MilStop®.

4. Treatment “CAB” = Max-In Calcium® + Aza-Direct® (I) + Max-In Boron®
Max-In Calcium® (Winfield Solutions LLC, St. Paul, Minnesota) and Max-In Boron® (Winfield Solutions LLC, St. Paul, Minnesota) were selected based on research exhibiting positive results for disease control and marketable fruit yield (Singh et al., 2007) and were applied alternately with one application consisting of Max-In Calcium® and Aza-Direct® (Gowan, Yuma, Arizona) labeled for aphids and mites as a mixture and then alternating with Max-In Boron® and Aza-Direct® for the next application.
5. Treatment “DAM” = Double Nickel® (F) + Aza-Direct® (I) + Mildew Cure® (F)
Double Nickel® (Certis, Columbia, Maryland) is labeled for control of powdery mildew and gray mold and was applied as a soil drench at planting and then combined with Aza-Direct® (Gowan, Yuma, Arizona) labeled for aphids and mites and along with Mildew Cure® (JH Biotech, Ventura, California) labeled for powdery mildew as a foliar application during the 2018 and 2019 harvest seasons.
6. Treatment “RGC” = Regalia® (F) + Grandevo® (I) + Cueva® (F)
Regalia® (Marrone Bio Innovations, Davis, California), Grandevo® (Marrone Bio Innovations, Davis, California), and Cueva® (Neudorff, Brentwood Bay, BC, Canada) were mixed together for foliar application. Regalia® is a plant extract designed to enhance natural defenses within strawberries for gray mold and powdery mildew, which was also applied as a soil drench at planting. Grandevo is a labeled insecticide to control aphids and mites. During 2018, it was determined that Cueva®, which is a Copper based fungicide labeled for gray mold and powdery mildew was causing phytotoxicity to the strawberry leaves and fruit. The rate used in 2018 was 7.5 L Cueva® to 378 L water,

after reducing the rate to 1.9 L Cueva® to 378 L water the toxicity issues did not reoccur for the 2019 season.

The acronym assigned to each treatment option will be used throughout this thesis to refer to the biopesticide combinations within the treatments. Root drench applications of treatments APA, ACM, DAM, and RGC were applied at the beginning of each season with applications of Actinovate, AmyProtec 42, Double Nickel, and Regalia per the labeled recommendations. The label recommendation of AmyProtect 42 was to only apply the pesticide three times during the season as a root or soil drench only. Five foliar applications of each biopesticide combination treatment were applied in 2018, along with an additional foliar application of Organic JMS Stylet-Oil to manage an out-of-control two-spotted spider mite (TSSM) population (Appx. D). Four foliar applications were applied during the 2019 season due to a later developing fruit set than the previous year (Appx. E). Six liters of water was used to apply the pesticides as foliar sprays to the plots within each treatment.

Experimental Design

This study was organized into a Split-Plot Randomized Block Design (Appx. F). The high tunnel fit three rows which was divided into six blocks. The split was between the two cultivars selected: Camino Real and Sweet Sensation. Each treatment combination was randomized within the two cultivars as the plots. Six plots contained one cultivar and one biopesticide combination. Each plot contained 12 plants arranged in a staggered pattern with 30 cm between each plant. Data was taken from eight plants, leaving two buffer plants on each end of the plot. The buffer plants were used so there was no cross-contamination from the different biopesticide treatment combinations. There were 72 plots total within the tunnel consisting of 864 plants in total with data being taken from 576 plants. SAS 9.2 software (SAS Institute, Cary,

NC) was used for statistical analysis using an ANOVA PROC GLM with significant differences determined using LS Means at an alpha level of 0.05.

Harvest Protocol

Harvest began in mid to late March and ended in mid-May for both seasons. In 2018, fruit onset began in late December but in 2019, fruit onset did not occur until early March. Each plot had an assigned JA Kitchens QUART Green Molded Pulp Fiber Berry / Produce Vented Basket (Amazon) that harvested fruit would be placed into. Fruit were harvested (depending on ripeness) one to two times per week during the harvest season. Each plot had eight plants that data collected from. Fruit ripeness was based on a fully red fruit with no white or green near the top. The fruit from the buffer plants were collected separately and not used for data analysis. The fruit collected for data analysis were sorted as marketable or unmarketable. Unmarketable fruit was determined from the perceived quality standard of the evaluator. Fruit could be unmarketable due to damage from disease, arthropods, nutrition, or physiological problems. After being sorted into marketable and unmarketable categories, all of the fruit in each category was weighed in grams using an Ohaus scale (Parsippany, NJ).

Environmental Conditions

Air temperature and relative humidity were recorded using a WatchDog weather logger (Spectrum Technologies, Aurora, IL) in the high tunnel during both seasons. Field weather conditions was collected from a WeatherUnderground data logger (UA Turf Science Program) located approximately 100 yards south from the study. Growing degree days (GDD) (base 50) (10°C) were calculated with the equation:

$$\text{GDD}_{50} = (T_h + T_l / 2) - 50$$

where T_h is the temperature high and T_l is the temperature low divided by 2 to get the average temperature minus the base temperature of 50 (Nugent, 2005). Daily light Integral (DLI, $\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$) was recorded for field conditions from a WeatherUnderground data logger. DLI was not recorded in the high tunnel. Relative humidity was recorded within the high tunnel, but not in the field. Field relative humidity was calculated using the August-Roche-Magnus approximation:

$$\text{RH} = 100 * (\text{EXP}((17.625 * \text{TD}) / (243.04 + \text{TD})) / \text{EXP}((17.625 * \text{T}) / (243.04 + \text{T})))$$

where TD = dew point temperature °C and T = temperature °C (University of Miami, 2020), so those results are an approximate and not exact numbers.

Economic Values of Biopesticide Treatments

Consideration was taken to calculate how much each biopesticide combination treatment cost to apply each season. The price for each biopesticide was calculated by taking the price \$ USD and amount (g/ml) of one unit sold, then finding the price of one gram or milliliter (\$/g(ml)) from the unit using this equation:

$$\$/\text{g}(\text{ml}) = \text{Product } \$ \text{ USD} / \text{Product Amount}$$

Then the actual amount of product applied to the treatment area ($\text{amt}/280\text{m}^2$) was then multiplied by the price of one gram or milliliter to find the price of the actual amount of product applied to the treatment area ($\$/\text{amt}/280\text{m}^2$) using this equation:

$$\$/\text{amt}/280\text{m}^2 = \text{amt}/280\text{m}^2 * \$/\text{g}(\text{ml})$$

Finally, the price of each biopesticide product applied to the treatment area was added together to find the cost per application and then multiplied by five to find the cost of five applications per season. None of these prices include the cost of labor, sprayer, PPE, or other inputs needed to apply the pesticides. Wage rates for field employees during peak strawberry harvest season (April 2019) in the Delta (Arkansas, Mississippi, and Louisiana) was \$12.37 per hour (USDA

NASS, 2019). The exact pesticide backpack sprayer used in this study is a Stihl SR 450 backpack sprayer, which costs \$699.95 (The Hardware Store, Fayetteville, AR). AmyProtec 42 is not for sale in the United States, so the cost for the ACM treatment was calculated for only Captiva® and MilStop®.

RESULTS

There was no significant effect of cultivar, biopesticide combination treatment or their interactions for number of total fruit, marketable fruit, and unmarketable fruit, in 2018 ($p>0.05$) or 2019 ($p>0.05$) (Table 1). Cultivar did not impact total fruit, or marketable fruit weights in 2018 or 2019 (Table 2). In 2019, there was a significant cultivar effect for unmarketable fruit weight ($p<0.05$) (Table 2). The cultivar Camino Real had lower unmarketable fruit weights than Sweet Sensation (445.66 g, 343.41g, respectively) ($p<0.05$) (Table 2). An effect of biopesticide combination treatments was significant for total and marketable fruit weights in 2018, but not in 2019. The RGC treatment had the lowest total fruit weight at 1,518.42 g, which was significantly different from Control (1,950.62 g), APA (1,845.53 g), ACM (1,883.84 g), and CAB (1,886.26 g) treatments; however, none of the treatments were significantly different from treatment DAM at 1,665.99 g ($p<0.05$) (Table 2). The Control treatment had higher marketable fruit weight (42.44 g), but was not significantly different from treatments ACM (819.64 g) and CAB (893.93 g). Treatment RGC had the lowest marketable weight at 632.19 g and was significantly different from treatments Control and CAB, but not from treatments ACM, APA (765.95 g), and DAM (729.71 g) ($p<0.05$) (Table 2). In 2018 there was a significant interaction between cultivar and biopesticide combination treatment for unmarketable weight ($p<0.05$) (Table 2). ‘Sweet Sensation’ with ACM treatment had the highest unmarketable weight (g) at 1139.17 g, but was not different from the other treatments: Control (986.99 g), APA (1065.76 g), CAB (1039.39 g),

DAM (976.02 g). The only treatment that was significantly different was the RGC treatment (745.07 g). For ‘Camino Real’, the only difference occurred between APA (1034.24 g) and Control (785.82 g) ($p < 0.05$) (Fig. 1). ‘Sweet Sensation’ by RGC had the lowest unmarketable fruit weight, but was not significantly different from ‘Camino Real, Control, DAM, ACM, CAB ($p < 0.05$) (Fig. 1). There was no significant effect for unmarketable yield by biopesticide treatment in 2019 (Table 2).

Biopesticide combination treatment APA was the most expensive treatment option at \$3.23 per one application and \$16.15 per five applications in a season (Table 3). The lowest treatment option was CAB (with the exception of the Control) at \$0.81 per one application and \$4.05 per five applications in a season (Table 3). The most expensive biopesticide is Aza-Direct at \$689.95 per 9.5 L and the most inexpensive products were Max-In Calcium and Max-In Boron at \$25 per 3.8 L (Table 3).

Daily light integral was recorded for ambient conditions for both harvest seasons in the field. The first season in 2017-2018 the DLI was within normal range during the critical vegetative and reproductive growth stages between February and May (Fig. 2). Whereas the DLI during the second season 2018-2019 was much lower than the normal DLI range. According to Faust and Logan (2018), the most recently updated high resolution maps of normal DLI range by the National Renewable Energy Laboratory in 2018 indicated that the normal DLI range for February is 20-25, March is 25-35, April is 35-40, and May is 40-45 (Table 3). In 2019, the recorded DLI was much lower than the normal DLI range with February at 11.2, March at 20.1, April at 28.9, and May at 31.3 (Fig. 2). The high tunnel in the first harvest season had the most cumulative growing degree days base 50 (GDD-50) at 1,973.36 by the end of the harvest season in May. Field GDD-50 in 2017-2018 was recorded at 1,677.5. The second harvest season also

indicates that the high tunnel had a higher amount of GDD-50 at 1,566.63 than the field at 1,458.8 by the end of the harvest season in May (Fig. 3). The lowest recorded relative humidity was in January 2018 for the field at 51.6%, while the high tunnel was at 83.6% (Fig. 4). The high tunnel in both seasons reached 79% relative humidity in February, which was the highest for the 2018 season but was already in decline for the 2019 season (Fig. 4).

DISCUSSION

Each harvest season consisted of collected data on fruit yield in terms of number and weight in grams. Yields were relatively low for both the 2018 and 2019 harvest seasons. On average, the yield per plant was 0.5 pounds of fruit per plant. Growers tend to expect 1.5 pounds of fruit per plant to be profitable. There were several issues that potentially contributed to lower yields. Soil samples indicated that levels of Nitrogen and Phosphorus were deficient in both years. Fertility levels of the macronutrients (Nitrogen, Phosphorus, and Potassium) were below the sufficient range for the 2019 trifoliolate and petiole samples, along with a deficiency in nitrate-nitrogen ($\text{NO}_3\text{-N}$). In the 2018 season, the high tunnel flooded multiple times. Much of the fruit was rotted due to the amount of flooding present, which is what caused the majority of unmarketable fruit for that season. Another problem was caused by raccoons that came in causing damage to the fruit. A much general possibility for lower yields is the orientation of the tunnel. A study conducted by Janke et al., (2017) stated that orientation of high tunnels below the latitudinal line of 40 degrees needs to be north to south so shading from the structure would not be an issue. The high tunnel used in this study is oriented east to west. In 2019, the daily light integral DLI during the months where most vegetative and reproductive growth occurs for Arkansas strawberry production (February-May) were lower than the normal average DLI. Cumulative growing degree days (GDD-50), were lower in 2019 for both the field and high

tunnel than in 2018. Since temperature and light are the two main factors in plant growth and development, this could be one of the reason yield was low during the 2019 season. Relative humidity was observed in the high tunnel for both the 2018 and 2019 season. Both seasons had similar results and did not indicate that relative humidity had an effect on marketability.

The strawberries were determined to be marketable or unmarketable by visual observation of each fruit. Fruit determined to be unmarketable would have physical defects, pest damage, or nutritional issues. During the 2018 harvest season, it was noted that the amount of unmarketable fruit greatly outnumbered and outweighed the amount of marketable fruit for each cultivar and treatment combination. In the 2019 harvest season the amount of marketable fruit outnumbered and outweighed the unmarketable fruit. For 2018, the percent of marketable fruit weight (g) for Camino Real was 48% and Sweet Sensation was 43% marketable. In 2019, there was an improvement for both cultivars in marketable fruit weight with Camino Real having 77% marketable and Sweet Sensation having 73% marketable fruit. Both cultivars produced similar amounts of total fruit weight (g) for each year. Lower yields in 2018 were attributed to flooding and raccoon damage. In 2019, fruit development was much later than the previous season. This is attributed to the lower DLI (quantity of light photons received) and GDD₁₀ (heat units accumulated above base growth temperature) received during the critical growing months for vegetative and subsequently reproductive growth stages.

None of the tested biopesticide combinations were shown to impact fruit yield in any appreciable way. Numerically, the control treatment (water) had the highest total fruit weight and marketable fruit weight for the 2018 harvest season. The control was not significantly different than the other treatment combinations and it was also noted that the CAB treatment had the second highest total and marketable fruit weight in 2018. During 2019, the CAB treatment had

the highest marketable and total fruit weight, while the control treatment had the second highest. There was toxicity to the plants in the RGC treatment for the 2018 season. It was determined that Cueva® was being applied at too high of a rate. The safety data sheet for Cueva® listed a range for the amount of product to be applied. After reducing the rate applied it was observed that there were no toxicity issues for the 2019 season. After reviewing the labels of Regalia®, Grandevo®, and Cueva® there was no indication that these three biopesticides would have a negative chemical reaction with the other. Furthermore, in Cueva's® label it is mentioned that copper toxicity is possible.

Biopesticides are an emerging technology that generates a small portion of the pesticide market. Conventional pesticides have a pest control efficiency level of 95% or greater most of the time as a one-step solution to pest problems, while biopesticides do not have the same efficacy level and should be utilized as an added tool for integrated pest management practices (Seiber et al., 2014). Even with reduced regulations from the EPA, not one biopesticide is listed in the top 10 pesticides used in California; however, this can be explained by conventional pesticides being used in high quantities while some biopesticides are only effective in small quantities (Seiber et al., 2014). The regulation standards to register biopesticides require a different set of data than conventional pesticides, which means consideration is taken into practicality of specific tests and only extra tests will be conducted if a potential risk to humans or the environment is detected (Leahy et al., 2014). The results of this study indicate that additional funds and research is needed for the continued development of biopesticides within the agriculture industry

The cost of each biopesticide combination treatment for one application is under \$3.50 and five applications per season was under \$20. However, the upfront cost of some of the

biopesticides can be too much initially such as Aza-Direct being priced just under \$700. The amount of product used and the area of the high tunnel where biopesticides are applied would depend on each individual case. Extra cost associated with labor, equipment and other inputs are not calculated within the cost reported.

CONCLUSION

During the two seasons of this research project, weather and climatic conditions had more of an impact on fruit development and marketability than the biopesticide treatment combinations. Even though the high tunnel offers slight protection from weather and climatic conditions, there were still problems that occurred due to flooding, temperature, and light conditions. The biological combination treatments were relatively cost effective due to the area sprayed and amount of times application occurred. However, there was not a clear indication that one biopesticide combination treatment was superior to the control (water) treatment or the CAB treatment consisting of nutrients. These results indicate that the biopesticide combinations should not be used by producers for improved fruit production or marketable yield of strawberries in high tunnels.

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TABLES AND FIGURES

Table 1. Effect of biopesticide combination applications on total, marketable and unmarketable fruit number on two strawberry cultivars: Camino Real and Sweet Sensation grown under high tunnel during the spring 2018 and 2019 seasons at the UAREC, Fayetteville, AR

	Total fruit number ^{z,y}	Marketable fruit number ^x	Unmarketable fruit number
2018			
Cultivar			
Camino Real	135.1	54.8	78.1
Sweet Sensation	128.9	43.4	83.1
<i>p</i> -value	0.6313	0.2904	0.4754
Biopesticide Combination			
Control ^w	147.6	66.6	78.2
APA	122.7	41.6	79.8
ACM	149.3	54.3	92.5
CAB	136.5	49.4	82.6
DAM	129.8	45.1	83.3
RGC	110.3	40.2	68.6
<i>p</i> -value	0.1661	0.1530	0.2472
<i>p</i> -value Cv X Biopest	0.2506	0.1710	0.6276
2019			
Cultivar			
Camino Real	105.6	73.0	31.9
Sweet Sensation	101.6	63.2	37.6
<i>p</i> -value	0.7255	0.1538	0.3392
Biopesticide Combination			
Control	106.9	69.9	36.0
APA	101.7	68.6	32.4
ACM	97.9	59.4	37.8
CAB	113.3	77.2	35.4
DAM	100.3	62.4	36.9
RGC	102.1	71.5	29.9
<i>p</i> -value	0.3778	0.2727	0.4001
<i>p</i> -value Cv X Biopest	0.4107	0.4632	0.6116

^zn=8 (number of plants per treatment, both cultivars)

^yMeans with different letter(s) for each attribute are significantly different ($p < 0.05$) using ls means significant difference.

^xAll fruit from each plot was assessed for marketability and was determined by the presence or lack of physical damage, arthropod/disease damage, and nutritional issues.

^wCombination of Biopesticides ID:

Control = Untreated Water Control; APA = Actinovate, PyGanic, Actinovate;

ACM = AmyProtec 42, Captiva, MilStop; CAB = Max-In Calcium, Aza-Direct, Max-In

Boron; DAM = Double Nickel, Aza-Direct, Mildew Cure; RGC = Regalia, Grandevo, Cueva

Table 2. Effect of biopesticide combination applications on total, marketable and unmarketable fruit weight (g) on two strawberry cultivars: Camino Real and Sweet Sensation grown under high tunnels during the spring 2018 and 2019 seasons at the UAREC, Fayetteville, AR.

	Total weight (g) ^{z,y}	Marketable weight (g) ^x	Unmarketable weight (g)
2018			
Cultivar			
Camino Real	1792.35	851.75	908.19
Sweet Sensation	1782.16	759.80	985.00
<i>p</i> -value	0.9578	0.5823	0.2583
Biopesticide Combination			
Control ^w	1950.62 a	1042.44 a	880.79 ab
APA	1845.53 a	765.95 bc	1049.96 a
ACM	1883.84 a	819.64 abc	1026.12 ab
CAB	1886.26 a	893.93 ab	978.39 ab
DAM	1665.99 ab	729.71 bc	920.85 ab
RGC	1518.42 b	632.19 c	837.33 b
<i>p</i> -value	0.0204	0.0362	0.0244
<i>p</i> -value Cv X Biopest	0.5152	0.3394	0.0307
2019			
Cultivar			
Camino Real	1549.98	1192.96	343.41 b
Sweet Sensation	1709.69	1245.28	445.66 a
<i>p</i> -value	0.1022	0.5215	0.0457
Biopesticide Combination			
Control	1688.70	1290.62	387.58
APA	1593.16	1213.55	369.44
ACM	1594.96	1123.48	452.91
CAB	1792.76	1375.68	393.45
DAM	1543.35	1103.48	424.65
RGC	1567.39	1227.34	330.84
<i>p</i> -value	0.6973	0.4038	0.2214
<i>p</i> -value Cv X Biopest	0.5053	0.3327	0.7707

^zn=8 (number of plants per treatment, both cultivars)

^yMeans with different letter(s) for each attribute are significantly different ($p < 0.05$) using ls means significant difference.

^xAll fruit from each plot was assessed for marketability and was determined by the presence or lack of physical damage, arthropod/disease damage, and nutritional issues.

^wCombination of Biopesticides ID:

Control = Untreated Water Control; APA = Actinovate, PyGanic, Actinovate;

ACM = AmyProtec 42, Captiva, MilStop; CAB = Max-In Calcium, Aza-Direct, Max-In

Boron; DAM = Double Nickel, Aza-Direct, Mildew Cure; RGC = Regalia, Grandevo, Cueva

Table 3. Cost comparison of biopesticide combinations per application and per five applications per season in 2018 and 2019 for two strawberry cultivars: Camino Real and Sweet Sensation grown under a HT at the UAREC in Fayetteville, AR.

Biopesticide	Trt ID	Product Amt	Product \$ USD	\$/g(ml)	amt/HT ²	\$/amt/HT ^z	\$/app	\$ five apps /season
Untreated	Control						0	0
Actinovate	APA	510g	103.77	0.2	5.1g	1.02		
PyGanic		907ml	49.5	0.05	44.25ml	2.21	3.23	16.15
AmyProtec 42 ^y		940ml	N/A	N/A	4.32ml	N/A		
Captiva	ACM	940ml	84.95	0.09	14.25ml	1.28		
MilStop		2300g	57.9	0.03	26.46g	0.79	2.07	10.35
Max-In Calcium		3800ml	25	0.01	14.7ml	0.15		
Aza-Direct	CAB	9500ml	689.95	0.07	9.3ml	0.65		
Max-In Boron		3800ml	25	0.01	0.0041ml	0.01	0.81	4.05
Double Nickel		9500ml	229.95	0.02	42.6ml	0.85		
Aza-Direct	DAM	9500ml	689.95	0.07	9.3ml	0.65		
Mildew Cure		19000ml	181.6	0.01	66ml	0.66	2.16	10.8
Regalia		3800ml	75	0.02	56ml	1.12		
Grandevo	RGC	2700g	149.95	0.06	20.4g	1.22		
Cueva		9500ml	114.95	0.01	28.5ml	0.29	2.63	13.15

^zArea for the high tunnel = 280m²

^yAmyProtec42 is not for sale in the United States

Product Amt = Amount of product sold as one unit

Product \$ USD = Price of one unit

\$/g(ml) = Price of one gram or milliliter from one unit

Amt/280m² = Actual amount of product applied to the treatment area of 280m²

\$/amt/280m² = Price of the actual amount of product applied to the treatment area

\$/app = Price of each biopesticide combination for one application

\$ five apps/season = Price of five applications of each biopesticide combination for the season

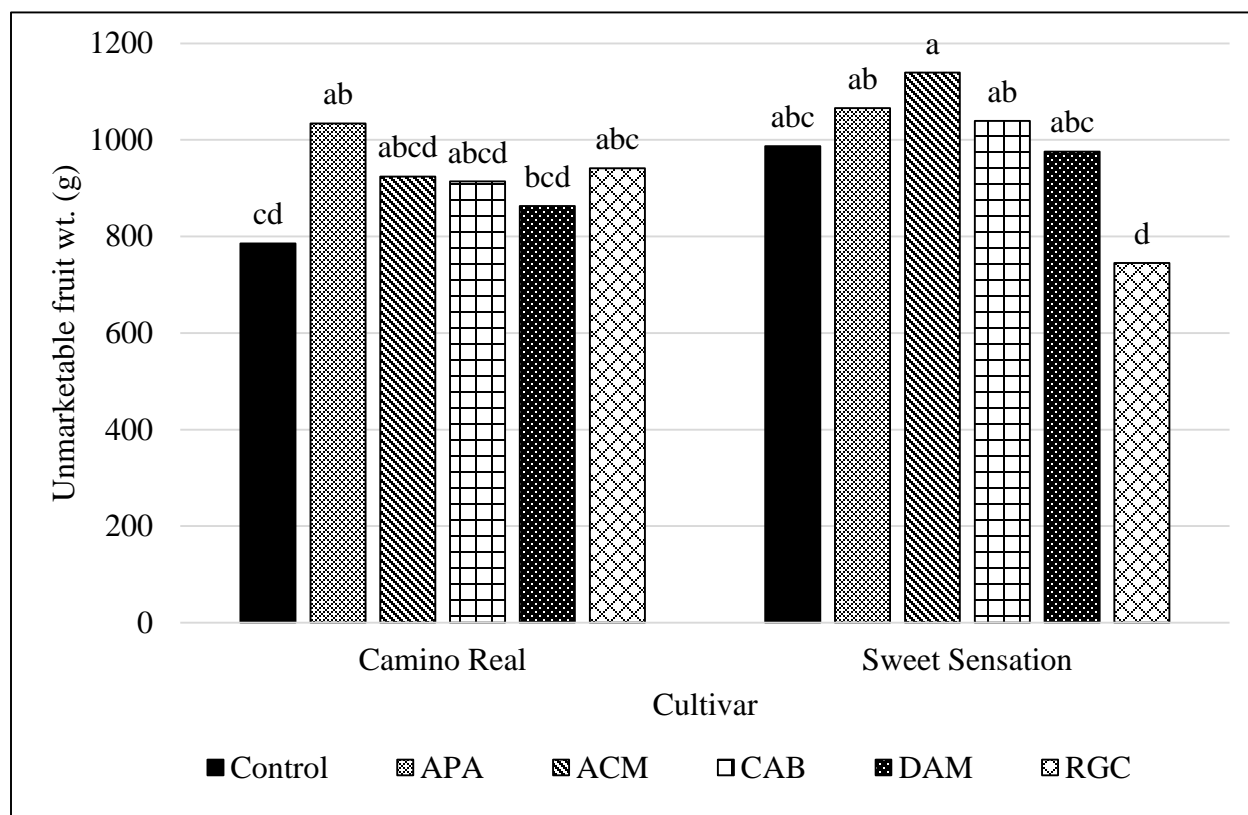


Fig. 1: Strawberry cultivar by biopesticide combination interaction for unmarketable fruit weight (g) for ‘Camino Real’ and ‘Sweet Sensation’ grown under high tunnels during the 2018 season at the UAREC in Fayetteville, AR.

Means with different letter(s) for each attribute are significantly different ($p < 0.05$) using ls means significant difference.

Combination of Biopesticides ID:

Control = Untreated Water Control; APA = Actinovate, PyGanic, Actinovate;

ACM = AmyProtec 42, Captiva, MilStop; CAB = Max-In Calcium, Aza-Direct, Max-In Boron;

DAM = Double Nickel, Aza-Direct, Mildew Cure; RGC = Regalia, Grandevo, Cueva

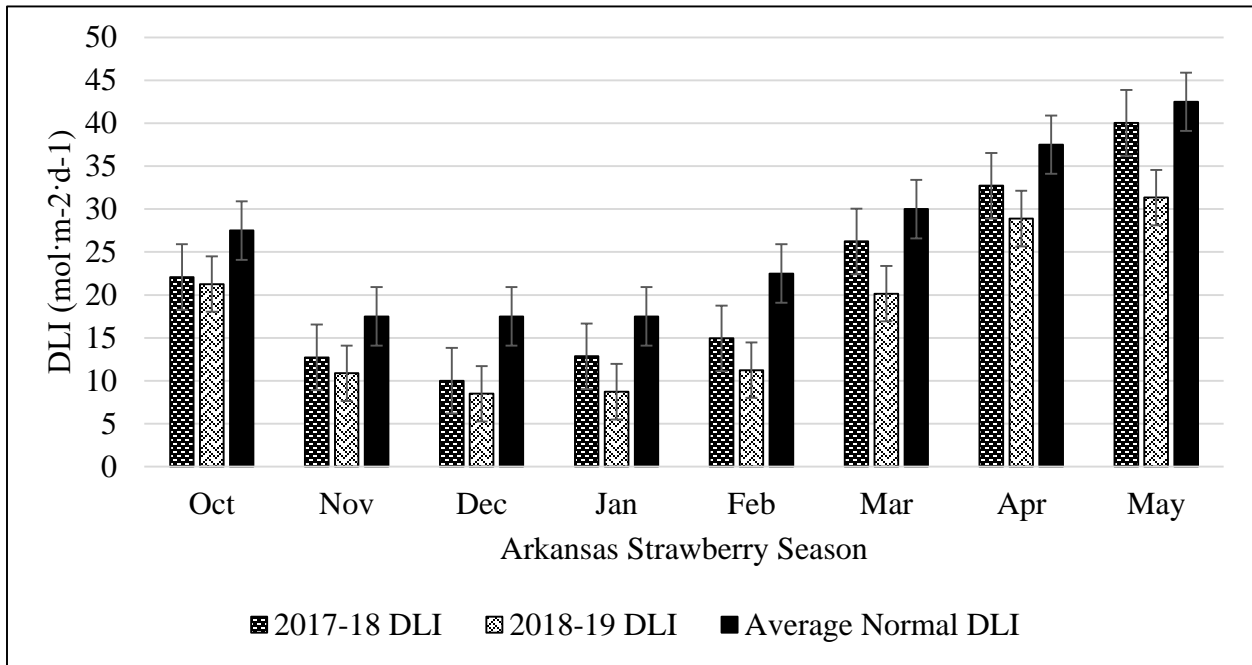


Fig. 2: Recorded DLI hours for the 2018 and 2019 strawberry growing season compared to average normal DLI levels. Data was taken from a WeatherUnderground data logger situated at the UAREC, Fayetteville, AR. Average DLI was obtained from Faust and Logan, 2018 and Torres and Lopez, 2012.

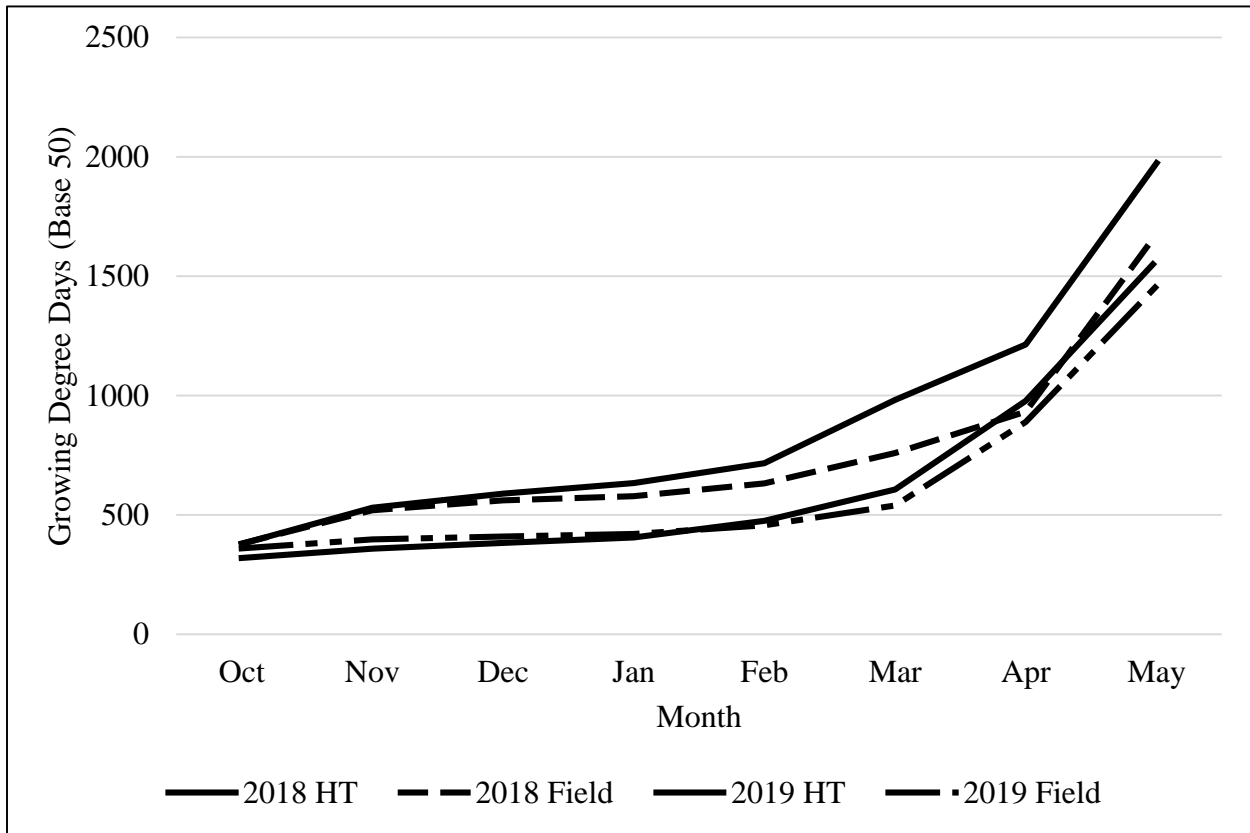


Fig. 3: Cumulative growing degree days base 50 (GDD-50) for the 2018 and 2019 strawberry season in high tunnel and field conditions. Data collected from a WatchDog weather logger located in the high tunnel and a WeatherUnderground data logger in the field located at the UAREC, Fayetteville, AR. Calculating cumulative GDD-50 equation: $DD = (\text{max. temp.} + \text{min. temp.}) / 2 - \text{base temp. (50)}$. Zero will be assigned for any DD with a negative number (Nugent, 2005).

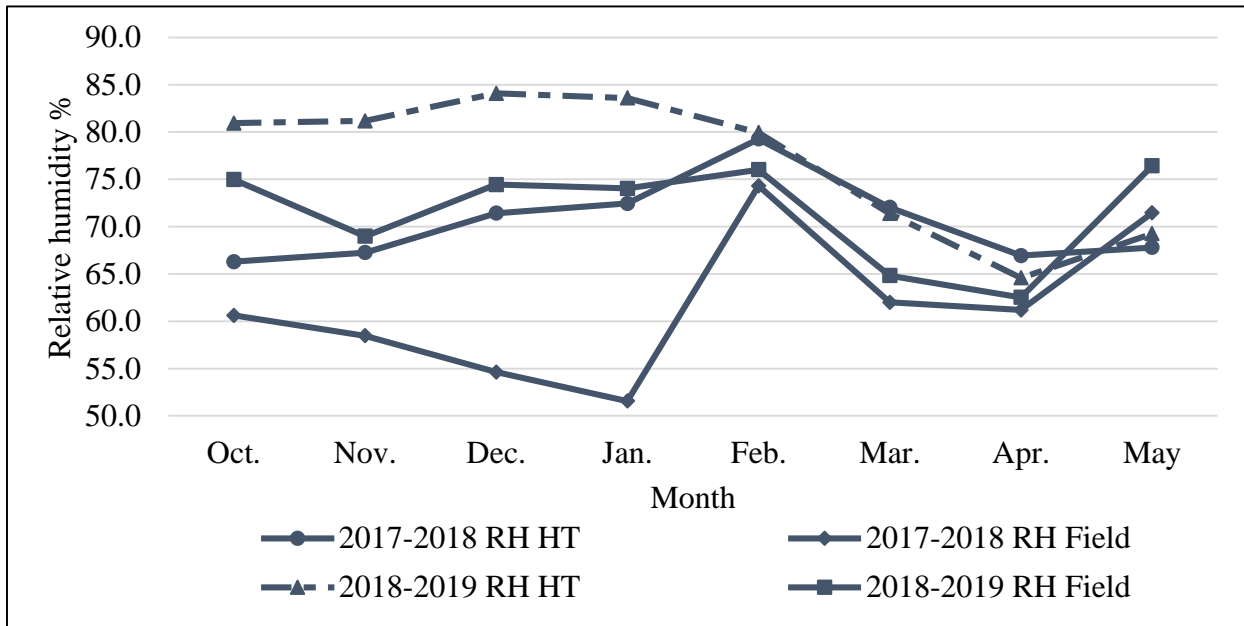


Fig. 4: Relative humidity % for the 2018 and 2019 strawberry season in high tunnel and field conditions. Data collected from a WatchDog weather logger located in the high tunnel and a WeatherUnderground data logger in the field located at the UAREC, Fayetteville, AR. Field relative humidity data is an approximate number calculated from the August-Roche-Magnus approximation: $RH = 100 * \frac{\text{EXP}((17.625 * TD) / (243.04 + TD))}{\text{EXP}((17.625 * T) / (243.04 + T))}$ where TD = dew point temperature °C and T = temperature °C (University of Miami, 2020).

CHAPTER 2

Effect of commercially available biopesticide combinations on control of four strawberry high tunnel pests: powdery mildew, gray mold, and two-spotted spider mites on strawberries grown in plasticulture production systems in high tunnels

ABSTRACT

Two strawberry cultivars (Camino Real and Sweet Sensation) and six different biopesticide combination treatments were evaluated for the control of three different strawberry high tunnel pests: powdery mildew (*Podosphaera aphanis* (Wallr.) U. Braun and S. Takam. (formerly *Sphaerotheca macularis* (Wall. Ex Fries) Jacz f. sp. *Fragariae* (Peries))), gray mold (*Botrytis cinerea*), and two-spotted spider mites (*Tetranychus urticae* (Koch) (Acari: Tetranychidae)) during the 2018 and 2019 harvest season. Treatments included untreated water control and five biopesticide treatment combinations: APA = Actinovate + PyGanic + Actinovate; ACM = AmyProtec + Captiva + MilStop; CAB = Max-In Calcium + Aza-Direct + Max-In Boron; DAM = Double Nickel + Aza-Direct + Mildew Cure; and RGC = Regalia + Grandevo + Cueva. In 2018, Sweet Sensation strawberry had significantly higher powdery mildew damaged fruit than Camino Real with less than 1% fruit damage in 2019. Significantly greater percentages of fruit were damaged by gray mold in plots of Camino Real treated with APA and RGC than plots treated with ACM, CAB, DAM that were similar to untreated control. In 2019, there were no significant effects by cultivar, biopesticide treatment combinations or interaction with cultivar. In 2018, Camino Real averaged significantly higher numbers of two-spotted spider mites and eggs per leaflet across three sampling dates than did Sweet Sensation. For all dates, the untreated control treatment plots of Camino Real had significantly higher cumulative mite days than did Sweet Sensation. By the last sample date, Camino Real plots treated with the biopesticide combination of DAM had the lowest numbers of mites per leaflet.

INTRODUCTION

Field strawberry (*Fragaria × ananassa* Duch.) production in Arkansas is difficult due to abiotic and biotic factors such as weather, disease and arthropod problems. The use of high tunnels can help mitigate some of this pressure by creating a physical barrier against precipitation, wind, and some pests. However, not all pests are excluded. Common pests in strawberry plasticulture production systems in high tunnels are powdery mildew, gray mold, two-spotted spider mites, and strawberry aphids.

Powdery mildew (*Podosphaera aphanis* (Wallr.) U. Braun and S. Takam. (formerly *Sphaerotheca macularis* (Wall. Ex Fries) Jacz f. sp. *Fragariae* (Peries))) is a fungal pathogen common in high tunnels due to increased humidity and favorable conditions (Demchak, 2009; Xiao et al., 2001). White, mycelial growth forms on fruit and foliar tissue that causes yield loss and poor fruit quality due to malformation or abortion (Maas, 1998; Peres and Mertely, 2009). Management of this disease relies on breeding efforts for powdery mildew resistance (Kennedy et al., 2013), disease-free plugs, and the implementation of a fungicide program (Maas, 1998).

Gray mold (*Botrytis cinerea*) is a common fungal pathogen that causes significant economic damage to strawberries (Petrasch, et al., 2019) and can potentially infect between 200 to upwards of 1,000 different plant host species (Elad et al., 2016; Williamson et al., 2007). Multiple methods of infection occur through direct contact, airborne conidia or infected flowers hosts through different inoculum sources (Williamson et al., 2007). Infection can occur in two ways, primary and secondary infection. Primary infection is initiated by conidia from adjacent infected plants (Jarvis, 1962) and then goes into an asymptomatic or quiescent phase until fruit ripens and then tissue is quickly destroyed (Williamson et al., 2007). Mechanisms of the asymptomatic or quiescent phase are not fully understood at this time (Petrasch et al., 2019).

Secondary infection does not have an asymptomatic phase, which causes immediate decay and is initiated by direct contact from infected leaves and fruit (Holz et al., 2007; Jarvis, 1962).

Methods of control include sanitation practices along with a developed fungicide program. At this time, there are no resistant strawberry cultivars to gray mold (Bestfleish et al., 2015; Bristow et al., 1986).

Two-spotted spider mites (*Tetranychus urticae* (Koch) (Acari: Tetranychidae)) is a microscopic arthropod that feeds on the underside of strawberry leaves by sucking the sap, which makes the leaves turn bronzed and mottled with severe infestations showing webbing (Bessin, 2019; Howell and Daugovish, 2013). Populations flourish when there is low soil moisture and high temperatures (White and Liburd, 2005). Economic threshold of two-spotted spider mites is five mites per leaflet (Burrack, 2017; Zalom et al., 2007) and injury threshold is measured in cumulative mite days (CMD) per leaflet (Hull and Beers, 1990). Mite management involves monitoring and scouting, sanitation, and natural enemies (White and Liburd, 2005; Howell and Daugovish, 2013). Insecticide applications are also a method of control; however, this method also reduces numbers of beneficial arthropods as well.

In 2018, organic food sales were reported at \$47.9 billion USD (OTA, 2019). The decision for producers to switch to organic production is greatly influenced by cost and other perceived risks including efficacy of biopesticides to control disease and pest (Veldstra et al., 2014). A rapidly developing technology for organic production is the use of biopesticides. Biopesticides are naturally occurring compounds used for pest control. North America approximately make up 45% of the demand for biopesticides (Bailey et al., 2010; Copping, 2014). However, biopesticides are still considered a niche market due to associated risks with cost, lack of efficacy, quality issues, short shelf-life, and lack of awareness (Arthurs, 2018;

Chandler et al., 2011; and Glare et al., 2012). Microbial, biochemical, semiochemicals, and plant-incorporated products are categories within the all-encompassing term biopesticides (Chandler et al., 2011; EPA, 2018). The classification of biopesticides is based on their compounds. Microbial biopesticides are composed of bacteria and other living organisms, biochemical biopesticides are comprised of products such as pyrethrins and natural oils, semiochemicals are products that include insect pheromones that can cause behavioral changes, and plant-incorporated products are closely related to genetically modified crops by genetic material being added to a plant for control or resistance (Arthurs and Dara, 2018; Chandler et al., 2011; Dunham, 2015; EPA, 2018; and Glare et al., 2012). Biopesticides are usually less toxic to the environment and humans (Damalas et al. 2018; Hubbard et al., 2014). Other benefits include that biopesticides are target specific and decompose quickly (EPA, 2018).

The objective of this study was to test the efficacy of selected commercially available biopesticides in controlling three strawberry pests grown in plasticulture production systems in high tunnels.

MATERIALS AND METHODS

Site Location

This study was conducted at the University of Arkansas Agriculture Research and Extension Center (UAREC) in Fayetteville, AR (Latitude: 36.1N; Longitude: 94.1W; USDA Cold Hardiness Zone 6b; AHS Heat Zone 7), during the 2018 and 2019 harvest seasons.

Strawberries were grown in an on-site, single bay ClearSpan™ Quonset-style high tunnel (FarmTek, Dyersville, Iowa) over Captina silt loam soil with a pH between 6.1 and 6.2 (Appx A). The tunnel was originally three separate tunnels but were put together to create a longer tunnel that is 6 m by 41.5 m and oriented East to West. The tunnel was covered with a single

layer, 6 mil UV treated polyethylene plastic with rolling down sidewall curtains and opening roll-up endwall doors for passive ventilation.

Production Management

All practices in this study were conducted according to the standards the Strawberry Production Guide for the Northeast, Midwest, and Eastern Canada (Pritts et al., 1998). Prior to planting the high tunnel, Bermuda grass (*Cynodon dactylon*) had grown in the space and was tilled during the summer of 2017 and 2018. In the summer of 2018, a cover crop of assorted cow peas was planted to deter the re-establishment of Bermuda grass. Irrigation was applied using sprinklers to have the ground ready for building beds. Three raised beds were constructed within the tunnel to be approximately 91 cm wide by 39.6 m long and 1.2 m apart. One mil black plastic mulch that was 1 m wide from Harris Seeds (Rochester, New York) and five mil t-tape was applied under the plastic mulch for irrigation (T-Tape Drip Tape, John Deere, Moline, Illinois). Landscape fabric was stapled between raised beds to deter weed establishment (Samtani et al., 2019). The plants were ordered from McNitt Growers (Carbondale, Illinois) in the summer of 2017 and 2018 for the 2018 and 2019 harvest period. The strawberry plugs were delivered by the last week of September and planting occurred during the first week of October for both years. For winter protection, low tunnels were constructed over the beds using cut rebar and thin poly tubing with baling string to keep the row cover floating above the plants. The row cover was a two mil white fabric, custom cut from BWI Industries (Texarkana, Arkansas). The tunnel was closed if the lowest predicted temperature was below 7°C and the row cover was applied if temperatures reached 1.5°C or lower. Soil analysis was conducted by the UA Agriculture Diagnostic Laboratory (UAADL) for both harvest seasons (Appx. A). The high tunnel was fertilized once during March 2018 with a Phosphorus focused fertilizer injected into the

irrigation system using a Dosatron® D25RE2 (QC Supply, Lincoln, Arkansas) because soil analysis confirmed there was a phosphorus deficiency. Trifoliolate and petiole samples were taken in 2019, which indicated that all macro and micronutrients were at sufficient levels (Appx. B). Samples were only taken in 2019 due to lack of funding to conduct the analysis in 2018. This study was treated as annual plasticulture production so the plants were removed from the ground at the end of harvest and the same protocol was used for the following year.

Cultivars

Two strawberry cultivars: Camino Real and Sweet Sensation were selected for this project. Camino Real was bred by Douglas V. Shaw and Kirk D. Larson at the University of California (Patent: USPP13079P2, Google Patents). It was patented in 2002 and is considered a short-day strawberry cultivar with good flavor and low unmarketable yields. ‘Camino Real’ was chosen because it is considered a widely used cultivar (A. McWhirt, personal communication). The cultivar, Sweet Sensation ‘Florida127’ was bred by Vance Whitaker at the University of Florida (Patent: USPP25574, University of Florida). It was released in 2013 and is comparable to two industry standards: ‘Florida Radiance’ and ‘Strawberry Festival’. ‘Sweet Sensation’ was chosen based on the recommendation that this cultivar is becoming increasingly popular within the organic strawberry community (B. McNitt, personal communication).

Combinations of Biopesticides

This study was conducted in conjunction with Texas A&M University project titled, “Evaluating organic pest control products for strawberries in combination with high and low tunnels for limited resource farmers in the Mid-South” funded by a Southern SARE R&E grant (LS16-275). As a collective group, the collaborators decided on which commercially available products would be tested within these studies. Several biopesticide products classified as

fungicides (F) and insecticides (I) were selected then arranged into six treatment combinations (Appx. C).

7. Treatment “Control” = foliar application of water.

Water was used as the control treatment because of a previous study conducted at the UA Fruit Research Station where data was skewed due to plants not receiving the same degree of wetness during pesticide application, causing less disease on unsprayed plants (Taunya Ernst, personal communication).

8. Treatment “APA” = Actinovate SP® (F) + PyGanic® (I) + Actinovate SP® (F)

Actinovate SP® (Novozymes, Franklinton, North Carolina) is labeled as both a root drench and foliar spray for preventative suppression/control of powdery mildew and gray mold on strawberries. PyGanic 1.4® (MKG, Minneapolis, Minnesota) is a commonly used insecticide for the control of two-spotted spider mites and strawberry aphids.

Actinovate SP® was applied as a root drench at planting and then mixed with PyGanic 1.4®, a pyrethrin that controls aphids and mites, as a foliar spray during the 2018 and 2019 harvest seasons.

9. Treatment “ACM” = AmyProtec 42 (F) + Captiva® (I) + MilStop® (F)

AmyProtec 42 (Andermatt Biocontrol, Grossdietwil, Switzerland) is not yet commercially available to American growers and is labeled as a soil or root drench only.

MilStop® (BioWorks, Victor, New York) is specifically labeled for powdery mildew on strawberries in the field and greenhouses. Captiva® (Gowan, Yuma, Arizona) is a registered insecticide for the control of mites used in combination as a foliar spray with MilStop®.

10. Treatment “CAB” = Max-In Calcium® + Aza-Direct® (I) + Max-In Boron®

Max-In Calcium® (Winfield Solutions LLC, St. Paul, Minnesota) and Max-In Boron® (Winfield Solutions LLC, St. Paul, Minnesota) were selected based on research exhibiting positive results for disease control and marketable fruit yield (Singh et al., 2007) and were applied alternately with one application consisting of Max-In Calcium® and Aza-Direct® (Gowan, Yuma, Arizona) labeled for aphids and mites as a mixture and then alternating with Max-In Boron® and Aza-Direct® for the next application.

11. Treatment “DAM” = Double Nickel® (F) + Aza-Direct® (I) + Mildew Cure® (F)

Double Nickel® (Certis, Columbia, Maryland) is labeled for control of powdery mildew and gray mold and was applied as a soil drench at planting and then combined with Aza-Direct® (Gowan, Yuma, Arizona) labeled for aphids and mites and along with Mildew Cure® (JH Biotech, Ventura, California) labeled for powdery mildew as a foliar application during the 2018 and 2019 harvest seasons.

12. Treatment “RGC” = Regalia® (F) + Grandevo® (I) + Cueva® (F)

Regalia® (Marrone Bio Innovations, Davis, California), Grandevo® (Marrone Bio Innovations, Davis, California), and Cueva® (Neudorff, Brentwood Bay, BC, Canada) were mixed together for foliar application. Regalia® is a plant extract designed to enhance natural defenses within strawberries for gray mold and powdery mildew, which was also applied as a soil drench at planting. Grandevo is a labeled insecticide to control aphids and mites. During 2018, it was determined that Cueva®, which is a Copper based fungicide labeled for gray mold and powdery mildew was causing phytotoxicity to the strawberry leaves and fruit. The rate used in 2018 was 7.5 L Cueva® to 378 L water, after reducing the rate to 1.9 L Cueva® to 378 L water the toxicity issues did not reoccur for the 2019 season.

The acronym assigned to each treatment option will be used throughout this thesis to refer to the biopesticide combinations within the treatments. Root drench applications of treatments APA, ACM, DAM, and RGC were applied at the beginning of each season with applications of Actinovate, AmyProtec 42, Double Nickel, and Regalia per the labeled recommendations. The label recommendation of AmyProtect 42 was to only apply the pesticide three times during the season as a root or soil drench only. Five foliar applications of each biopesticide combination treatment were applied in 2018, along with an additional foliar application of Organic JMS Stylet-Oil to manage an out-of-control two-spotted spider mite (TSSM) population (Appx. D). Four foliar applications were applied during the 2019 season due to a later developing fruit set than the previous year (Appx. E). Six liters of water was used to apply the pesticides as foliar sprays to the plots within each treatment.

Experimental Design

In 2018 and 2019, this study was organized into a Split-Plot Randomized Block Design (Appendix 2). Three raised beds were laid within the high tunnel and were divided into six blocks. The split was between the two cultivars selected: Camino Real and Sweet Sensation. Each treatment combination was randomized within the two cultivars as the plots. Six plots contained one cultivar and one biopesticide combination. Each plot contained 12 plants arranged in a staggered pattern with 30 cm between each plant. The number of mites per leaflet were averaged from six leaflets per plot and disease incidence was assessed from eight plants per plot, leaving two buffer plants on each end of the plot. The buffer plants were used so there was no cross-contamination from the different biopesticide treatment combinations. There were 72 plots total within the tunnel consisting of 864 plants in total with data being taken from 576 plants. SAS 9.2 software (SAS Institute, Cary, NC) was used for statistical analysis using an ANOVA

PROC GLM with significant differences determined using LS Means at an alpha level of 0.05. Disease incidence was given a severity rating; however, after running a frequency test it was determined that the number of non-diseased fruit greatly outweighed diseased fruit and analysis would not converge for the separated ratings. So all diseased fruit was combined and the data was analyzed as affected and not affected fruit categories.

Pesticide Application Protocol

Each biopesticide combination was applied to 12 plots. The area of the 12 plots totaled 280 m². Rate of application was followed by each biopesticide label and calculated for the appropriate area of 280m². Using a 14 L backpack sprayer (Stihl SR 450®, Virginia Beach, Virginia), a test application of water concluded that six liters of solution would cover all 12 plots. Before applying each treatment, it was advised that a test was conducted using a flask with water and appropriate amounts of each pesticide to ensure there was no negative chemical reaction. The exact concentration of each biopesticide treatment is given in Appx. C.

Disease Assessment

During harvest the strawberries were assessed for disease incidence. Ten strawberries were randomly selected from each plot. Each fruit was assessed visually for powdery mildew (*Podosphaera aphanis*) and gray mold (*Botrytis cinerea*). A rating scale by Palmer, S. (2007) was used to assess the disease incidence. The severity of the disease was assessed by examining each fruit individually and rating the fruit with a score from 0 (no disease) to 5 (dead, rotten fruit) (Appx. G). Information from North Carolina State University's Strawberry Diagnostic Key were used to properly identify disease symptoms (NC State Extension, 2017). Disease incidence was given a severity rating; however, after running a frequency test it was determined that the number of non-diseased fruit greatly outweighed diseased fruit and analysis would not converge

for the separated ratings. Therefore, all diseased fruit was combined and the data was analyzed as affected and not affected fruit categories.

Arthropod Assessment

Six leaflets were collected from each of the 72 plots, three times during the seasons. Each collection was done one day prior to a pesticide application. A mite brush machine was used to brush mites and eggs from sampled leaves onto a circular glass plate with a film of immobilizing soap and water solution. Macmillian and Costello (2015) found that a mite brush machine was much more effective than visual counts on the leaves. The brushed mites on the glass plate was then set on a disk so the mites and aphids could be counted using a microscope. The disk contained 50 sections, divided into 25 colored sections and 25 uncolored sections in an alternating pattern (Fig. 1).

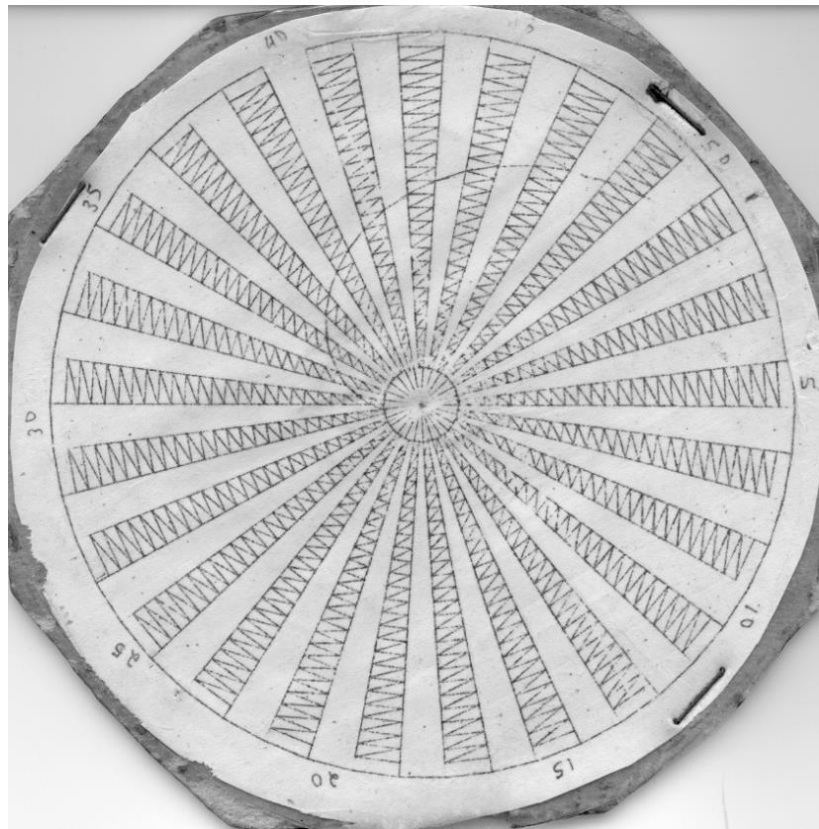


Figure 5. Sampling grid for counting mites brushed onto a round glass plate.

The following sequential sampling program was used to estimate number of brushed mites per leaflet using the average number of two-spotted spider mites (TSSM) per section to determine when to stop scanning plate sections: 0-50 TSSM = 20 sections counted; 51-100 TSSM = 15 sections counted; 101-150 TSSM = 10 sections counted; and 151-200 TSSM = 5 sections counted. The formula for calculating the mean number of mites per leaflet is:

$$\text{Mites per Leaflet} = M * 50.48 / S * L$$

where M is the total number of mites counted, 50.48 is a correction factor to convert the portion of counted sections and uncounted sections, center and outer ring (Johnson, personal communication), S is the number of sections counted and L is the number of leaflets brushed.

Another method of assessing cumulative feeding damage by two-spotted spider mite populations is through cumulative mite days (CMD) calculated as follows (Hull and Beers 1990):

$$\text{CMD} = \sum 0.5(P_a + P_b)D_{a-b}$$

where P_a and P_b are the mean number of mites per leaflet for sampling date a and b and D is the amount of days between sampling dates.

RESULTS

Powdery Mildew: In 2018, percentages of fruit damaged by powdery mildew were significantly affected by cultivar, but no effects were due to biopesticide combination treatments or interaction with cultivar (Table 4). The strawberry cultivar Sweet Sensation had 20.5% of fruit damaged by powdery mildew that was significantly greater than the 9.3% damaged fruit on Camino Real. In 2019, there were no significant effects by cultivar, biopesticide treatment combinations or interaction with cultivar.

Gray mold: In 2018, percentages of fruit damaged by gray mold was significantly affected by biopesticide treatment combinations and interaction with cultivar (Table 4), but there

was no cultivar effect. Significantly greater percentages of fruit were damaged by gray mold in Camino Real fruit treated with APA (28%) = RGC (35%) than 17% or less of fruit with gray mold in ACM = CAB = DAM = untreated control (Figure 5). In 2019, there were no significant effects by cultivar, biopesticide treatment combinations or interaction with cultivar.

Two-spotted spider mites (TSSM): In 2018, average numbers of TSSM and eggs per leaflet across three sampling dates were significantly affected by cultivar. Camino Real had 75.6 TSSM and 143.1 TSSM eggs per leaflet that were significantly greater than the 25.3 TSSM and 56 TSSM eggs per Sweet Sensation leaflet (Table 5). There were significant effects on cumulative mites days (CMD) per leaflet due to interaction of biopesticide combination treatments with cultivar. The untreated control treatment plots had 1154.4 CMD per Camino Real leaflet that was significantly higher than 138.9 CMD per Sweet Sensation leaflet (Figure 6). All biopesticide combination treatments were equal to untreated control for Camino Real (ranged from 300 to 900 CMD) and Sweet Sensation (ranged from 190-300 CMD). In 2018 and 2019, biopesticide combination treatments did not significantly differ in numbers of TSSM or TSSM eggs per leaflet. In 2019, there were no significant effects on CMD by cultivar, biopesticide treatment combinations or interaction with cultivar.

Effects by date: In 2018, numbers of TSSM per leaflet (Table 6) or TSSM eggs per leaflet (Table 7) for given sampling date were similar across both cultivars and all biopesticide combination treatments. The sampling date with the highest numbers of TSSM per leaflet was on 4 April for Camino Real with treatment CAB while the lowest count was for Camino Real on 13 May with the DAM treatment (Table 8). There was no significant interaction of the biopesticide combination treatments by cultivar and date for the number of TSSM eggs per leaflet in 2019 (Table 9).

DISCUSSION

Strawberry high tunnel pest populations relate to the environmental conditions provided by the high tunnel. Powdery mildew is often associated with subsequent infestations of TSSM within high tunnels (Asalf et al., 2012) where a favorable microclimate is created by the increased relative humidity that promotes powdery mildew development and provides a physical protection from rain and wind for the development of TSSM (Demchak, 2009; Ingwell et al., 2017; Xiao et al., 2001).

In 2019, the rate of fruit infection from both powdery mildew and gray mold was not enough to cause economic damage. Sweet Sensation did have more fruit damage than Camino Real for 2018. In 2019, cultivar, biopesticide combination treatment and their interaction did not show the significant differences due to low infections of 1% and 2% powdery mildew and gray mold damaged fruit, respectively. Prokkola and Kivijarvi (2007) stated that their experiment had low levels of disease incidence as well and also found that biopesticides did not have a significant effect when compared to the untreated control. The study concluded with cultural control methods being an important factor in organic strawberry production (Prokkola and Kivijarvi, 2007).

It was observed in 2018 that cultivar did have a significant effect in TSSM populations. ‘Camino Real’ in 2018 had a significantly larger population of TSSM than Sweet Sensation. It was noted that the population of TSSM decreased over time for Camino Real and increased for Sweet Sensation. However, the population of TSSM on Sweet Sensation was still lower than Camino Real throughout time, but both cultivars had TSSM populations greater than the economic threshold of 5 mites per leaflet (Burrack et al., 2017; Zalom et al., 2007). In 2019, Sweet sensation still had lower counts of TSSM than Camino Real, but the two cultivars were

not significantly different as from the year before. However, TSSM per leaflet were only above economic threshold for both cultivars for the first sampling date, yet during the second and third date the number of TSSM per leaflet was below the economic threshold. Date was significantly different for 2019 where the population of mites had begun to take hold but then decreased significantly over time. Number of TSSM eggs per leaflet reflect the pattern for number of TSSM per leaflet indicating the high populations for 2018 and the lower populations for 2019. In 2019, TSSM per leaflet was below the economic threshold, with that information growers would decide not to apply pesticides. The biopesticide combinations did not indicate that one combination had an advantage over the other for the control of TSSM populations. Most research into biological control of TSSM is with the use of predatory arthropods. Attai et al., (2013) suggests that essential oils have potential in managing mite populations; however, more information needs to be directed toward the improvement of extraction methods, mode of action, cost, and toxicity toward predatory mites and other beneficial arthropods.

Each biopesticide has an individual label and recommendation for best control method. For this study, the biopesticides were applied as a combination, which negated the recommendations for the best method of control. Most pesticides are recommended during different phenological stages of plant development (i.e. bloom, pre-harvest, post-harvest), which is accelerated within a high tunnel. Some biopesticides are microbial based with an active ingredient such as bacteria, which could require a certain temperature for application. An example in this study includes the biopesticide Actinovate®, which is ineffective when temperatures reach below 7°C yet it had to be applied within the combination for consistency in this project.

CONCLUSION

The combinations of biopesticides did not have a clear significant effect on disease incidence or arthropod infestation levels. Pest scouting and mechanical control methods such as sanitation by removing diseased or damaged fruit and leaves had a greater effect in managing infection incidence and infestation levels. The fruit and plants were naturally infected/infested and not inoculated with the disease or arthropods. The 2018 season had higher levels of powdery mildew, gray mold and TSSM than the 2019 seasons. These results, in part, could be caused by environmental conditions. Powdery mildew specifically thrives in humid environments and two-spotted spider mites thrive in a protected environment from rain and wind, which the high tunnel is capable of creating and maintaining. Since the plants were not inoculated, this meant that conditions were adequate for disease and TSSM development in 2018, but not for 2019. Based on the results of this study, it is not recommended for growers to use any of the biopesticide combinations tested. Lastly, it is recommended that these biopesticides should be tested individually and follow the methods of best control to indicate the true efficacy of each biopesticide.

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TABLES AND FIGURES

Table 4. Effect of biopesticide combination applications on percent fruit disease (powdery mildew and gray mold) damage on two strawberry cultivars: Camino Real and Sweet Sensation grown under high tunnels during spring 2018 and spring 2019 seasons at the UAREC, Fayetteville, AR.

	Powdery Mildew		Gray Mold	
	% Damaged fruit ^{z,y,x}	% Healthy fruit	% Damaged fruit	% Healthy fruit
2018				
Cultivar				
Camino Real	9.3 b	90.7 a	18.7	81.3
Sweet Sensation	20.5 a	79.5 b	17.7	82.3
<i>p-value</i>	0.006		0.71	
Biopesticide Combination				
Control ^w	14.9	85.1	17.2	82.8
APA	14.1	85.9	24.2	75.8
ACM	12.9	87.1	14.5	85.5
CAB	13.8	86.2	16.8	83.2
DAM	15.8	84.2	16.4	83.6
RGC	12.3	87.7	21.3	78.7
<i>p-value</i>	0.4426		<0.0001	
<i>p-value Cv X Biopest</i>	0.1843		<0.0001	
2019				
Cultivar				
Camino Real	0.2	99.8	0.7	99.3
Sweet Sensation	0.4	99.6	0.3	98.7
<i>P-value</i>	ns		0.11	
Biopesticide Combination				
Control	0.8	99.2	1.7	98.3
APA	0.3	99.7	1.5	98.5
ACM	0	100	0.9	99.1
CAB	0.1	99.9	0.6	99.4
DAM	0.5	99.5	0.6	99.4
RGC	0	100	1.1	98.9
<i>p-value</i>	ns		0.23	
<i>p-value Cv X Biopest</i>	ns		0.64	

^zn=10 (Number of randomly selected fruit per plot)

^yMeans followed by the same letter in each column are not significantly different at $p=0.05$

^xFruit was assessed using a rating scale from 0 (no disease) to 5 (dead/rotted fruit).

^wCombination of Biopesticides ID: Control = Untreated Water Control; APA = Actinovate, PyGanic, Actinovate; ACM = AmyProtec 42, Captiva, MilStop; CAB = Max-In Calcium, Aza-Direct, Max-In Boron; DAM = Double Nickel, Aza-Direct, Mildew Cure; RGC = Regalia, Grandevo, Cueva

Table 5. Effect of biopesticide combination applications on numbers of two-spotted spider mites (TSSM) per leaflet, TSSM eggs per leaflet, and cumulative mite days on two strawberry cultivars: Camino Real and Sweet Sensation grown under high tunnel during the spring 2018 and 2019 season at the UAREC, Fayetteville, AR.

	Mites per leaflet ^{z,y,x} (6 leaflets)	Eggs per leaflet (6 leaflets)	Cumulative mite days
2018			
Cultivar			
Camino Real	75.6 a	143.1 a	613.7
Sweet Sensation	25.3 b	56.0 b	230.6
<i>p-value</i>	0.01	0.02	0.02
Biopesticide Combination			
Control ^w	50.2	87.9	400.6
APA	37.0	84.8	359.9
ACM	53.8	103.8	441.9
CAB	61.9	137.7	477.9
DAM	46.0	91.8	401.3
RGC	24.6	52.7	232.05
<i>p-value</i>	0.052	0.16	0.11
<i>p-value</i> Cv X Biopest	0.12	0.74	0.04
2019			
Cultivar			
Camino Real	3.7	30.7	85.4
Sweet Sensation	3.4	26.8	60.7
<i>p-value</i>	0.69	0.33	0.11
Biopesticide Combination			
Control	3.1	26.9	66.8
APA	4.9	40.6	73.4
ACM	3.9	30.6	84.4
CAB	3.6	21.5	100.8
DAM	2.7	24.4	45.2
RGC	3.9	31.6	73.8
<i>p-value</i>	0.14	0.12	0.21
<i>p-value</i> Cv X Biopest	0.27	0.23	0.17

^zn=6 (number of leaflets collected from each plot)

^yMeans followed by the same letter within each column are not significantly different at $p=0.05$

^xThe following formula was used to estimate the number of mites (eggs) per leaflet:

(total # of mites(eggs) counted)*(50.48) / (# of sections)*(# of leaves)

^wCombination of Biopesticides ID:

Control = Untreated Water Control; APA = Actinovate, PyGanic, Actinovate;

ACM = AmyProtec 42, Captiva, MilStop; CAB = Max-In Calcium, Aza-Direct, Max-In Boron;

DAM = Double Nickel, Aza-Direct, Mildew Cure; RGC = Regalia, Grandevo, Cueva

Table 6. Sample date effect of biopesticide combination applications on numbers of two-spotted spider mites (TSSM) per leaflet on two strawberry cultivars: Camino Real and Sweet Sensation grown under high tunnel during the spring 2018 season at the UAREC, Fayetteville, AR.

Biopesticide	Trt ID:	Rate	13 March	27 March	10 April	13 March	27 March	10 April
			_{z,y,x}	Camino Real			Sweet Sensation	
Untreated	Control		230.6	140.3	93.0	8.9	20.8	28.5
Actinovate	APA	5g - 7.6 L/H ₂ O (d)	62.5	43.9	38.6	14.7	43.4	38.4
PyGanic		170-340g - 378.5L/H ₂ O (f)						
AmyProtec 42	ACM	0.03-0.8L - 3.8 L/H ₂ O	116.2	94.5	98.6	17.7	38.9	32.4
Captiva		207-355mL - 0.4 ha						
MilStop	CAB	0.5-1L - 378.5L/H ₂ O	108.6	128.2	105.5	31.5	25.8	47.4
Max-In Calcium		0.5kg -0.4ha						
Aza-Direct	DAM	2.0kg Ca ha ⁻¹ spray ⁻¹	63.4	71.7	64.4	24.5	34.4	38.6
Max-In Boron		0.5-1L - 0.4ha						
Double Nickel	RGC	0.5-5.7L - 378.5L/H ₂ O (f)	37.1	33.6	35.3	14.5	13.4	25.7
Aza-Direct		0.2-2L - 378.5L /H ₂ O (d)						
Mildew Cure		0.5-1L - 0.4ha						
Regalia		3.8L - 378.5L/H ₂ O						
Grandevo		19-38mL - 3.8L/H ₂ O						
Cueva		1-1.4kg - 378.5L/H ₂ O						
		7.6L - 378.5L/H ₂ O						
<i>p</i> -value			0.31					

^zn=6 (number of leaves collected from each plot)

^yMeans followed by the same letter within same row (across dates in same year) are not significantly different at P=0.05

^xThe following formula was used to estimate the number of mites (eggs) per leaflet:

(total # of mites(eggs) counted)*(50.48) / (# of sections)*(# of leaves)

Table 7. Sample date effect of biopesticide combination applications on numbers of two-spotted spider mite eggs per leaflet on two strawberry cultivars: Camino Real and Sweet Sensation grown under high tunnel during the spring 2018 season at the UAREC, Fayetteville, AR.

Biopesticide	Trt ID:	Rate	13 March	27 March	10 April	13 March	27 March	10 April
			_{z,y,x}	Camino Real			Sweet Sensation	
Untreated	Control		266.9	214.3	97.9	18.4	62.1	72.7
Actinovate	APA	5g - 7.6 L/H ₂ O (d)	90.3	127.1	129.1	23.4	85.6	125.3
PyGanic		170-340g - 378.5L/H ₂ O (f) 0.03-0.8L - 3.8 L/H ₂ O						
AmyProtec 42	ACM	207-355mL - 0.4 ha	224.1	182.3	142.3	25.4	100.9	83.9
Captiva		0.5-1L - 378.5L/H ₂ O						
MilStop	CAB	0.5kg -0.4ha	219.5	251.4	120.2	78.0	111.3	118.1
Max-In Calcium		2.0kg Ca ha ⁻¹ spray ⁻¹						
Aza-Direct	DAM	0.5-1L - 0.4ha	84.2	191.9	125.7	35.9	101.5	80.9
Max-In Boron		0.3mL - 0.4ha						
Double Nickel	RGC	0.5-5.7L - 378.5L/H ₂ O (f)	54.8	185.8	95.3	10.6	47.6	43.4
Aza-Direct		0.2-2L - 378.5L /H ₂ O (d)						
Mildew Cure		0.5-1L - 0.4ha						
Regalia		3.8L - 378.5L/H ₂ O						
Grandevo		19-38mL - 3.8L/H ₂ O						
Cueva		1-1.4kg - 378.5L/H ₂ O						
		7.6L - 378.5L/H ₂ O						
<i>p</i> -value			0.67					

^zn=6 (number of leaves collected from each plot)

^yMeans followed by the same letter within same row (across dates in same year) are not significantly different at P=0.05

^xThe following formula was used to estimate the number of mites (eggs) per leaflet:

(total # of mites(eggs) counted)*(50.48) / (# of sections)*(# of leaves)

Table 8. Sample date effect of biopesticide combination applications on numbers of two-spotted spider mites (TSSM) per leaflet on two strawberry cultivars: Camino Real and Sweet Sensation grown under high tunnel during the spring 2019 season at the UAREC, Fayetteville, AR.

Biopesticide	Trt ID:	Rate	4 April	22 April	13 May	4 April	22 April	13 May
			_{z,y,x}	Camino Real			Sweet Sensation	
Untreated	Control		9.7 abcd	7.1 abcde	0.5 e	11.3 abcd	2.4 abcde	0.9 cd
Actinovate	APA	5g - 7.6 L/H ₂ O (d)	10.6 abcd	6.9 abcde	1.7 bcde	11.5 abcd	3.7 abcde	2.9 abcde
PyGanic		170-340g - 378.5L/H ₂ O (f)						
AmyProtec 42	ACM	207-355mL - 0.4 ha	14.0 ab	5.6 abcde	1.3 cde	12.9 abc	3.7 abcde	0.8 e
Captiva		0.5-1L - 378.5L/H ₂ O						
MilStop	CAB	0.5kg -0.4ha	21.3 a	2.6 abcde	0.9 de	9.6 abcd	4.6 abcde	0.8 e
Max-In Calcium		2.0kg Ca ha ⁻¹ spray ⁻¹						
Aza-Direct	DAM	0.5-1L - 0.4ha	3.2 abcde	4.1 abcde	0.6 e	15.1 ab	0.9 e	3.5 abcde
Max-In Boron		0.3mL - 0.4ha						
Double Nickel	RGC	0.5-5.7L - 378.5L/H ₂ O (f)	14.2 ab	4.4 abcde	2.3 abcde	9.3 abcd	4.2 abcde	0.7 e
Aza-Direct		0.2-2L - 378.5L /H ₂ O (d)						
Mildew Cure		0.5-1L - 0.4ha						
Regalia		3.8L - 378.5L/H ₂ O						
Grandevo		19-38mL - 3.8L/H ₂ O						
Cueva		1-1.4kg - 378.5L/H ₂ O						
		7.6L - 378.5L/H ₂ O						
<i>p</i> -value			0.002					

^zn=6 (number of leaves collected from each plot)

^yMeans followed by the same letter within same row (across dates in same year) are not significantly different at P=0.05

^xThe following formula was used to estimate the number of mites (eggs) per leaflet:

(total # of mites(eggs) counted)*(50.48) / (# of sections)*(# of leaves)

Table 9. Sample date effect of biopesticide combination applications on numbers of two-spotted spider mite eggs per leaflet on two strawberry cultivars: Camino Real and Sweet Sensation grown under high tunnel during the spring 2019 season at the UAREC, Fayetteville, AR.

Biopesticide	Trt ID:	Rate	4 April ^{z,y,x}	22 April	13 May	4 April	22 April	13 May
			Camino Real			Sweet Sensation		
Untreated	Control		77.7	61.9	3.0	60.8	34.8	12.5
Actinovate	APA	5g - 7.6 L/H ₂ O (d)	68.1	46.6	12.9	71.7	50.5	30.2
PyGanic		170-340g - 378.5L/H ₂ O (f) 0.03-0.8L - 3.8 L/H ₂ O						
AmyProtec 42	ACM	207-355mL - 0.4 ha	76.8	67.2	14.5	84.5	38.5	3.4
Captiva		0.5-1L - 378.5L/H ₂ O						
MilStop	CAB	0.5kg -0.4ha	56.8	27.5	7.8	62.0	28.9	4.6
Max-In Calcium		2.0kg Ca ha ⁻¹ spray ⁻¹						
Aza-Direct	DAM	0.5-1L - 0.4ha	46.3	34.2	9.3	70.9	13.2	15.1
Max-In Boron		0.3mL - 0.4ha						
Double Nickel	RGC	0.5-5.7L - 378.5L/H ₂ O (f)	96.6	54.7	13.7	93.4	30.5	4.9
Aza-Direct		0.2-2L - 378.5L /H ₂ O (d)						
Mildew Cure		0.5-1L - 0.4ha						
Regalia		3.8L - 378.5L/H ₂ O						
Grandevo		19-38mL - 3.8L/H ₂ O						
Cueva		1-1.4kg - 378.5L/H ₂ O						
		7.6L - 378.5L/H ₂ O						
<i>p</i> -value			0.12					

^zn=6 (number of leaves collected from each plot)

^yMeans followed by the same letter within same row (across dates in same year) are not significantly different at P=0.05

^xThe following formula was used to estimate the number of mites (eggs) per leaflet:

(total # of mites(eggs) counted)*(50.48) / (# of sections)*(# of leaves)

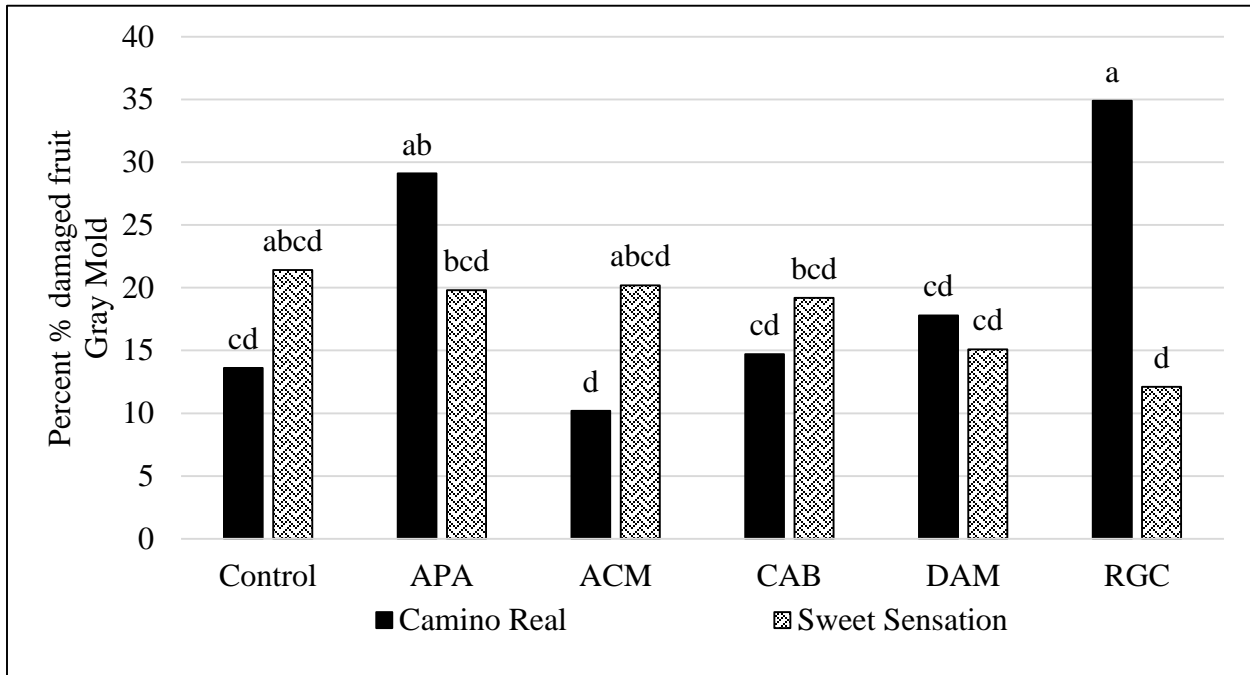


Fig. 6: Cultivar and biopesticide combination interaction for percent % damaged fruit by gray mold on two strawberry cultivars: Camino Real and Sweet Sensation and six biopesticide combinations grown under high tunnels during the spring 2018 season at the UAREC, Fayetteville, AR.

Combination of Biopesticides ID:

Control = Untreated Water Control; APA = Actinovate, PyGanic, Actinovate;

ACM = AmyProtec 42, Captiva, MilStop; CAB = Max-In Calcium, Aza-Direct, Max-In Boron;

DAM = Double Nickel, Aza-Direct, Mildew Cure; RGC = Regalia, Grandevo, Cueva

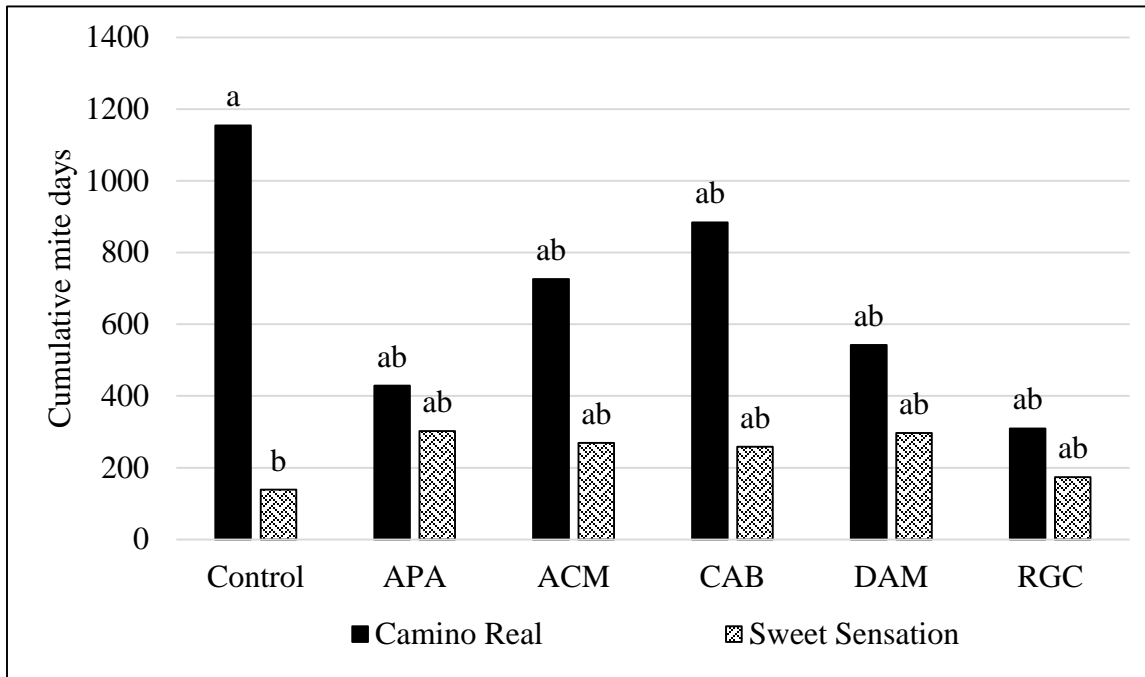


Fig. 7: Cultivar by biopesticide combination interaction for cumulative mite days by date in 2018 on two strawberry cultivars: Camino Real and Sweet Sensation grown under high tunnel at the UAREC in Fayetteville, AR.

Combination of Biopesticides ID:

Control = Untreated Water Control; APA = Actinovate, PyGanic, Actinovate;

ACM = AmyProtec 42, Captiva, MilStop; CAB = Max-In Calcium, Aza-Direct, Max-In Boron;

DAM = Double Nickel, Aza-Direct, Mildew Cure; RGC = Regalia, Grandevo, Cueva

OVERALL CONCLUSION

An Evaluation of Biopesticide Combinations on Yield Performance and Disease/Arthropod Control of Strawberries Grown in High Tunnel Plasticulture Production Systems in Arkansas.

OVERALL CONCLUSIONS

During the course of this study, it became evident that none of the combinations of biopesticides displayed an overall advantage over another. Numerically speaking, the control (water) treatment and the CAB treatment had the highest total and marketable fruit weight for both seasons. The cost of each biopesticide combination was relatively cost effective for the amount applied to the treatment area (280m²). The reported costs did not include labor and other inputs. The biopesticide AmyProtect 42 is not registered or for sale in the United States, so that particular combination only included those biopesticides sold in the United States. Other than the control (water) treatment, treatment CAB was the most cost effective at \$0.81 USD per one application and \$4.05 USD per five applications within a season. Since CAB had numerically higher total and marketable yields than the other treatments this is a very cost effective treatment for producers. Even with the added protection of a high tunnel, environmental pressure still had an effect on plant growth and fruit development due to flooding, temperature and light. DLI and GDD₁₀ were lower in 2019 than in 2018. Temperature and light are important for strawberry production because those factors can determine if a plant will produce stolons (other daughter plants) or branch crowns which produce fruit. Within an annual plasticulture production system, the production of stolons is discouraged because the plants for that season are replaced with new ones each year so fruit production is the main goal.

Yields were relatively low for both seasons at 230 g/plant. Environmental factors such as the high tunnel being flooded multiple times during the 2018 season and lower DLI and GDD₁₀ in 2019 caused lower yields. Another factor that could have caused reduced yields was due to the orientation of the high tunnel. The orientation of the tunnel is important in terms of shading and providing optimal light conditions.

None of the combinations of biopesticides had a clear advantage controlling disease incidence or arthropod infestation levels. Scouting and sanitation by removing diseased or damaged fruit and leaves had a greater effect in controlling incidence and infestation levels. The fruit and plants were naturally infected/infested and not inoculated with the disease or arthropods. The 2018 season had higher levels of powdery mildew, gray mold and TSSM than the 2019 seasons. These results, in part, could be caused by environmental conditions. Powdery mildew specifically thrives in humid environments and two-spotted spider mites thrive when rain or moisture is present on the plants, which the high tunnel is capable of creating and maintaining this environment. Since the plants were not inoculated, this meant that conditions were adequate for disease and TSSM development in 2018, but not for 2019. The recommendation to see the actual efficacy of these combinations of biopesticides is to inoculate the plant in a controlled laboratory setting where conditions are perfect for the disease and arthropod development and environmental factors will not contribute.

APPENDIX

Appx. A: Soil analysis conducted by UAADL for strawberry high tunnel in the 2018 and 2019 harvest season at the UAREC in Fayetteville, AR.

Year	pH	EC	P	K	Ca	Mg	S	Na	Fe	Mn	Zn	Cu	B
2018	6.1	198.0	40.5	110.1	1381.1	88.1	17.8	22.7	104.2	105.5	2.7	6.3	0.1
2019	6.2	412.5	78.8	166.8	1633.6	107.4	58.5	28.1	132.6	170.3	5.1	8.7	0.3

Appx. B: Trifoliolate and Petiole analysis conducted by UAADL for strawberry high tunnel in the 2019 harvest season at the UAREC in Fayetteville, AR.

	ID	mg/kg												id	NO ₃ -N		
		N	P	% K	Ca	Mg	S	Na	Fe	Mn	Zn	Cu	B				
Trifoliolate	Camino Real	Control	2.16	0.16	1.17	1.54	0.23	0.13	23	216	85	16	5.4	25	Petiole	2120	
		APA	1.60	0.12	0.93	1.82	0.30	0.11	44	431	99	20	6.9	43		2235	
		ACM	2.30	0.15	1.25	1.45	0.23	0.14	28	171	67	14	4.8	23		2185	
		CAB	2.04	0.14	1.12	1.79	0.31	0.13	27	291	104	18	5.6	29		2285	
		DAM	2.31	0.15	1.16	1.55	0.25	0.14	30	302	86	17	5.3	24		2475	
		RGC	2.32	0.17	1.33	1.48	0.23	0.15	32	211	69	16	5.4	25		2720	
	Sweet Sensation	Control	2.36	0.18	1.30	1.74	0.33	0.15	21	136	87	17	5.4	31		1690	
		APA	2.17	0.16	1.35	1.82	0.36	0.14	24	154	73	15	4.6	34		1985	
		ACM	2.28	0.15	1.38	1.76	0.32	0.15	27	186	70	16	5.0	31		2315	
		CAB	2.10	0.15	1.50	1.63	0.31	0.13	30	182	62	14	4.1	34		2110	
		DAM	2.42	0.17	1.26	1.80	0.32	0.16	22	242	92	17	5.4	30		1870	
		RGC	1.92	0.13	1.16	1.76	0.31	0.14	52	269	77	15	5.0	36		1970	
	Sufficient Range		2.0-2.8	0.25-0.4	1.5-2.5	0.7-1.7	0.3-0.5	0.4-0.6		60-250	50-200		20-50	6-20		30-70	3000-5000

Appx. C: Biofungicide (F) and Bioinsecticide (I) combination treatments used on two strawberry cultivars: Camino Real and Sweet Sensation grown under high tunnel at the UAREC in Fayetteville, AR during the 2018 and 2019 harvest season.

Biopesticide	Trt. ID	Active Ingredient	Dosage
Untreated	Control	Water	
Actinovate (F)		<i>Streptomyces lydicus</i> WYEC 108	5 g per 7.6 L/H ₂ O (drench)
PyGanic (I)	APA	Pyrethrins	0.03-0.8 L per 3.8 L/H ₂ O
Actinovate (F)		<i>Streptomyces lydicus</i> WYEC 108	170-340g per 378.5L/H ₂ O (foliar)
AmyProtec 42 (F)		<i>Bacillus amyloliquefaciens</i> FZB 42	207-355 mL per 0.4 ha
Captiva (I)	ACM	Capsicum oleoresin extract + garlic oil + soybean oil	0.5-1L per 378.5L/H ₂ O
MilStop (F)		Potassium bicarbonate	0.5 kg per 0.4 ha
Max-In Calcium		<i>Calcium</i>	2.0 kg Ca ha ⁻¹ spray ⁻¹
Aza-Direct	CAB	<i>Azadirachtin</i>	0.5-1 L per 0.4 ha
Max-In Boron		<i>Boron</i>	0.3 mL per 0.4 ha
Double Nickel (F)		<i>Bacillus amyloliquefaciens</i> D747	0.5-5.7L per 378.5L/H ₂ O (foliar) 0.2-2L per 378.5L /H ₂ O (drench)
Aza-Direct (I)	DAM	<i>Azadirachtin</i>	0.5-1L per 0.4 ha
Mildew Cure (F)		Cotton oil + Garlic oil	3.8L per 378.5L/H ₂ O
Regalia (F)		Extract of <i>Reynoutria</i> <i>sachalinensis</i>	19-38 mL per 3.8L/H ₂ O
Grandevo (I)	RGC	<i>Chromobacterium</i> <i>subtsugae</i> strain PRAA4-1 & spent fermentation media	1-1.4kg per 378.5L/H ₂ O
Cueva (F)		Copper octanoate	1.9-7.6L to 378.5L/H ₂ O

Appx. D: Treatment application dates and method for the 2018 season for strawberry cultivars Camino Real and Sweet Sensations grown under a HT at the UAREC in Fayetteville, AR

Biopesticide	Trt ID	Root Drench			Foliar Spray					
		1 Oct.	2 Mar.	5 Apr.	19 Jan.	14 Feb.	2 Mar.	14 Mar.	5 Apr.	26 Apr.
Water	Control				X		X	X	X	X
Actinovate	APA	X			X		X	X	X	X
PyGanic							X	X	X	X
AmyProtec 42		X	X	X						
Captiva	ACM						X	X	X	X
MilStop					X		X	X	X	X
Max-In Calcium					X			X		X
Aza-Direct	CAB					Organic JMS Stylet-Oil	X	X	X	X
Max-In Boron							X		X	
Double Nickel		X			X		X	X	X	X
Aza-Direct	DAM						X	X	X	X
Mildew Cure					X		X	X	X	X
Regalia		X			X		X	X	X	X
Grandevo	RGC						X	X	X	X
Cueva					X		X	X	X	X

Appx. E: Treatment application dates and method for the 2019 season for strawberry cultivars Camino Real and Sweet Sensations grown under a HT at the UAREC in Fayetteville, AR

Biopesticide	Trt ID	Root Drench			Foliar Spray			
		3 Oct.	16 Feb.	5 Apr.	16 Feb.	18 Mar.	5 Apr.	23 Apr.
Water	Control				X	X	X	X
Actinovate	APA	X			X	X	X	X
PyGanic					X	X	X	X
AmyProtec 42		X	X	X				
Captiva	ACM				X	X	X	X
MilStop					X	X	X	X
Max-In Calcium					X		X	
Aza-Direct	CAB				X	X	X	X
Max-In Boron						X		X
Double Nickel		X			X	X	X	X
Aza-Direct	DAM				X	X	X	X
Mildew Cure					X	X	X	X
Regalia		X			X	X	X	X
Grandevo	RGC				X	X	X	X
Cueva					X	X	X	X

Appx. F: Split-Plot Randomized Block Design of two strawberry cultivars: Camino Real (CR) and Sweet Sensation (SS) with six combinations of biopesticides in a high tunnel system with three raised beds separated into six blocks that were split by the two cultivars located at the UAREC in Fayetteville, AR for the 2018 and 2019 harvest season.

Block 1			Block 3			Block 5		
Plot #	Cultivar	Trt ID	Plot #	Cultivar	Trt ID	Plot #	Cultivar	Trt ID
1	SS	CAB	25	CR	RGC	49	SS	APA
2	SS	Control	26	CR	ACM	50	SS	CAB
3	SS	DAM	27	CR	CAB	51	SS	DAM
4	SS	APA	28	CR	Control	52	SS	ACM
5	SS	RGC	29	CR	APA	53	SS	Control
6	SS	ACM	30	CR	DAM	54	SS	RGC
7	CR	Control	31	SS	CAB	55	CR	ACM
8	CR	DAM	32	SS	APA	56	CR	DAM
9	CR	RGC	33	SS	Control	57	CR	Control
10	CR	CAB	34	SS	ACM	58	CR	RGC
11	CR	ACM	35	SS	RGC	59	CR	CAB
12	CR	APA	36	SS	DAM	60	CR	APA
Block 2			Block 4			Block 6		
Plot #	Cultivar	Trt ID	Plot #	Cultivar	Trt ID	Plot #	Cultivar	Trt ID
13	CR	DAM	37	SS	RGC	61	CR	Control
14	CR	APA	38	SS	DAM	62	CR	APA
15	CR	RGC	39	SS	APA	63	CR	ACM
16	CR	Control	40	SS	CAB	64	CR	CAB
17	CR	ACM	41	SS	ACM	65	CR	RGC
18	CR	CAB	42	SS	Control	66	CR	DAM
19	SS	Control	43	CR	Control	67	SS	CAB
20	SS	APA	44	CR	DAM	68	SS	DAM
21	SS	RGC	45	CR	ACM	69	SS	Control
22	SS	ACM	46	CR	APA	70	SS	RGC
23	SS	DAM	47	CR	CAB	71	SS	ACM
24	SS	CAB	48	CR	RGC	72	SS	APA

Combination of Biopesticides ID:

Control = Water control

APA = Actinovate® + PyGanic® + Actinovate®

ACM = AmyProtec 42 + Captiva® + MilStop®

CAB = Max-In Calcium® + Aza-Direct® + Max-In Boron®

DAM = Double Nickel® + Aza-Direct® + Mildew Cure®

RGC = Regalia® + Grandevo® + Cueva®

Appx. G: Visual scale for assessing disease incidence in harvested fruit.

Score	Description
0	No visible symptoms
1	Few small patches on a fruit
2	Patches covering 25% on a fruit
3	Patches covering 50% on a fruit
4	Patches covering 75% on a fruit
5	Dead, rotten fruit

Table derived from Palmer, S., Ph.D. dissertation, 2007. Strawberry powdery mildew: epidemiology and the effect of host nutrition on disease. Page 56.

Appx. H: ANOVA table of interaction between cultivar and biopesticide combination treatment on the number of total, marketable, and unmarketable fruit, measured during the 2018 and 2019 harvest season. n = 8

Factor	DF	Fruit number variables (P < F)		
		Total	Marketable	Unmarketable
2018				
Cultivar	1	0.6313	0.2904	0.4754
Biopesticide combination	5	0.1661	0.1530	0.2472
Cv X Biopest	5	0.2506	0.1710	0.6276
2019				
Cultivar	1	0.7255	0.1538	0.3392
Biopesticide combination	5	0.3778	0.2727	0.4001
Cv X Biopest	5	0.4107	0.4632	0.6116

Appx. I: ANOVA table of interaction between cultivar and biopesticide combination treatment on the weight of total, marketable, and unmarketable fruit (g), measured during the 2018 and 2019 harvest season. n = 8

Factor	DF	Fruit weight variables (P < F)		
		Total	Marketable	Unmarketable
		2018		
Cultivar	1	0.9578	0.5823	0.2583
Biopesticide combination	5	0.0244	0.0362	0.0204
Cv X Biopest	5	0.5152	0.3394	0.0307
		2019		
Cultivar	1	0.1022	0.5215	0.0457
Biopesticide combination	5	0.6973	0.4038	0.2214
Cv X Biopest	5	0.5053	0.3327	0.7707

Appx. J: ANOVA table of interaction between cultivar and biopesticide combination treatment on disease incidence of powdery mildew and gray mold, measured during the 2018 and 2019 harvest season. n = 10

Disease variables (P < F)			
Factor	DF	Powdery Mildew	Gray Mold
2018			
Cultivar	1	0.0059	0.7065
Biopesticide combination	5	0.4426	<.0001
Cv X Biopest	5	0.1843	<.0001
2019			
Cultivar	1	ns	0.1094
Biopesticide combination	5	ns	0.2310
Cv X Biopest	5	ns	0.6368

Appx. K: ANOVA table of interaction between cultivar, biopesticide combination treatment, and date on the number of two-spotted spider mites per leaflet, TSSM eggs per leaflet, and cumulative mite days, measured during the 2018 and 2019 harvest season. n = 6

TSSM variables (P < F)

Factor	DF	TSSM per leaflet	TSSM eggs per leaflet	Cumulative Mite Days
2018				
Cultivar	1	0.0062	0.0247	0.0188
Biopesticide combination	5	0.0525	0.1639	0.1061
Cv X Biopest	5	0.1154	0.7374	0.0442
Cv X Biopest X Date	10	0.3084	0.6699	0.2034
2019				
Cultivar	1	0.6990	0.3274	0.1084
Biopesticide combination	5	0.1425	0.1167	0.2106
Cv X Biopest	5	0.2726	0.2328	0.1745
Cv X Biopest X Date	10	0.0024	0.1216	ns