Evaluation of winter cover crops and biological control products to manage *Meloidogyne incognita* and insect pest damage in organic sweetpotatoes

by

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Abstract

There is a need to develop effective organic integrated pest management practices for sweetpotatoes. Our findings indicated that the combination of BotaniGard 22WP, Triple Threat Entomopathogenic Nematodes, and Majestene significantly reduced insect damage to sweetpotatoes under field conditions. The winter cover crops elbon rye and the mix containing crimson clover, daikon radish, elbon rye, and wheat resulted in lowered soil *M. incognita* populations compared with leguminous winter cover crops like field peas and crimson clover. Total insect pest damage was similar across winter cover crops, but lowest following crimson clover in North Carolina and black oats in Alabama. Soil health values measured by the Solvita CO_2 Burst test were elevated following the winter cover crop mixes compared with the single winter cover crop treatments, indicating that the mixes stimulate higher maximal biological activity, which relates to increased soil health. Overall, the integration of biological control products and winter cover crops shows promise for enhancing organic sweetpotato production while promoting soil health and sustainability.

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Introduction and Review of Literature

Sweetpotato

Sweetpotato (*Ipomoea batatas* (L.) Lam.) is considered the seventh most important food crop in the world (FAO, 2023). It is a member of the *Convolvulaceae* family, which includes morning glory (Mukhopadhyay et al., 2011). This vegetable crop is primarily cultivated for its starchy, nutrient-dense roots which are consumed as human food, animal feed, or for industrial uses (Truong et al., 2018). In addition, sweetpotatoes can be used to produce ethanol, industrial alcohol, and biofuel (Bovell-Benjamin, 2010). In 2022, the United States harvested 132,200 acres (53,419 hectares) of sweetpotatoes which was worth $598,424,000 (USDA, 2023). American sweetpotato production primarily occurs in the southeastern region of the United States with Alabama, Louisiana, Mississippi, and North Carolina producing 75% of domestic sweetpotatoes (Johnson et al., 2015). In 2017, Alabama harvested 2,178 acres of sweetpotatoes (USDA, 2023). These plants are cultivated around the world and are best suited to tropical and subtropical regions (Mukhopadhyay et al., 2011). They are vegetatively propagated and often grown from vine cuttings called slips (Jatala and Bridge, 1990). Sweetpotatoes prefer sandy, well-drained soils which allow their storage roots to expand without restriction (Mukhopadhyay, et al., 2011). Due to its well-developed root system which can reach deep into the soil profile, sweetpotato is considered a drought tolerant vegetable which can be grown in low-fertility soils (Fuentes and Chujoy, 2009, Jatala and Bridge 1990). These desirable agronomic qualities make this crop well-suited to low-input production systems, which are common throughout the developing world. Additionally, sweetpotatoes are highly nutritious, and the orange and yellow-fleshed cultivars are recognized as good sources of carotene and vitamin C (Mukhopadhyay, et

al., 2011). Interestingly, their vibrant colors make sweetpotatoes useful as food colorants used during food processing (Mukhopadhyay, et al., 2011).

Organic agriculture

The number of organic farms in the United States has increased to fulfill growing consumer demand for certified organic products (Lotter, 2003). Vegetables, which include sweetpotatoes, were grown on 57% of American organic farms in 1997, and they continue to be popular crops for organic farmers today (Greene and Kremen, 2003). Since synthetic pesticide use is prohibited in organic systems, farmers employ ecologically based pest and fertility management strategies (Greene and Kremen, 2003). However, these strategies are often less effective than conventional practices for pest and fertility management. In fact, organic farmers cite "effectiveness of organically allowable inputs and methods" as a key production constraint on their operations and identify the need for further research into organic management of insect and nematode pests (Walz, 1999). Despite these limitations, many farmers continue to produce organic products which can command a higher market price (Lotter, 2003). Other benefits of organic farming include improved soil health, lower pesticide usage, greater ecological harmony, and reduced energy input (Greene and Kremen, 2003).

Plant-parasitic nematodes

Parasitic nematodes are obligate parasites that require living plant hosts to develop and reproduce (Oka et al., 2000). These nematodes attach themselves to plant roots where they withdraw water and nutrients, resulting in a loss of plant vigor and a reduction in plant health (Oka et al., 2000). In fact, an international survey of crop losses of up to 10.2% in sweetpotato yield were the direct result of plant-parasitic nematodes (Palomares- Rius and Kikuchi, 2013).

The most economically important plant-parasitic nematodes affecting sweetpotato are *Meloidogyne* spp., *Pratylenchus* spp., *Rotylenchulus reniformis* (Linford and Oliveira 1940), and *Ditylenchus destructor* (Thorne 1945) (Jatala and Bridge, 1990). To a lesser extent, *M. arenaria* (Neal) Chitwood, *M. hapla* Chitwood, and *M. javanica* (Treub) Chitwood (Overstreet, 2013) also affect sweetpotato. Root-knot nematodes (*Meloidogyne* spp.) are the most serious pest in this crop because they can drastically reduce sweetpotato yield and quality (Kim and Yang, 2019). Looking forward, plant-parasitic nematodes could become even more problematic pests. In fact, researchers in Asia have reported increased plant-parasitic nematode damage to sweetpotato due to climate change and global warming (Kim and Yang, 2019).

Meloidogyne incognita

The southern root-knot nematode (*Meloidogyne incognita* (Kofoid and White)) is a sedentary endoparasitic nematode with a broad host range of >3000 plant species, including sweetpotato (Abad et al., 2003). This nematode causes both yield and quality reducing damage in sweetpotato in addition to many other economically important cropping systems (Jatala and Bridge, 1990). The primary visual symptom of root-knot nematode infection is the presence of root galls on host plant root systems (Bird, 1974). Higher *M. incognita* population densities are associated with more, and larger, galls (Overstreet, 2013). The degree of galling is variable and associated with sweetpotato cultivar, with some cultivars producing many large galls, and others producing smaller more inconspicuous galls (Overstreet, 2013). These galls damage the root system by interrupting nutrient and water uptake (Abad et al., 2003). Nematode infection results in weak, stunted, and low-yielding plants which display symptoms of nutrient deficiencies (Abad et al., 2003). In sweetpotato, infection with *M. incognita* results in smaller plants, earlier maturity, lower yields, and fewer marketable potatoes (Agu, 2004). *Meloidogyne incognita* also

interacts with pathogens like *Fusarium* spp. and *Pseudomonas solanacearum* (Smith) forming disease complexes that can cause wilting and early death (Jatala and Bridge, 1990). All races of *M. incognita* can infect sweetpotato at varying degrees, but in the United States, race 1 and race 3 are most common in sweetpotato (Jatala and Bridge, 1990; Clark and Moyer, 1988; Taylor and Sasser, 1985). The nematodes invade both feeder and storage roots at similar rates (Jatala and Bridge, 1990). Infected feeder roots are typically shorter and have fewer secondary roots and root hairs (Jatala, 1991). *Meloidogyne incognita* infection also causes longitudinal cracking and galling of the sweetpotato root surface, reducing quality and marketability (Overstreet, 2009). Cracks that begin early in the growing season are long and extend deep within the periderm, while cracks that begin in the later season are shorter and shallower (Jatala, 1991). Especially in the later season, this cracking allows pathogenic organisms to enter the sweetpotato root, causing rot (Lawrence et al., 1986). *Meloidogyne incognita* females can be observed embedded within the sweetpotato root when roots are sliced at 0.5 cm. These females are often surrounded by necrotic root tissue and egg masses (Lawrence et al., 1986). To begin its life cycle, a second stage juvenile (J2) *M. incognita* pierces the root tip with its stylet and begins feeding (Abad et al., 2003). The nematode feeding causes the root cells to differentiate into specialized giant cells as the J2 nematode establishes its permanent feeding site and becomes sedentary within the root. These giant cells are multinucleate and can be up to 100x larger than typical plant root cells, and groups of these swollen cells comprise the root galls (Moens et. al., 2009). Feeding at the giant cells for about 14 days, the J2 withdraws nutrients from the plant, enlarges, and molts to become a third stage juvenile (J3). After another 4-6 days, an additional molt occurs and the nematode develops into a fourth stage juvenile (J4), which develops into an adult female. The females then lay eggs in a protective gelatinous matrix which can be found on the surface of galled roots or

embedded within the root tissue. The nematodes undergo one molt inside the egg to develop from a J1 into a J2 which then emerges when environmental conditions are favorable, and the reproductive-infective cycle repeats. (Moens et. al., 2009). A life cycle is completed approximately every 24 days depending on temperature and moisture. During this time, nematodes continue to colonize the developing sweetpotatoes with 4-6 generations per growing season.

Insect pests

Two hundred seventy species of insects are considered pests of sweetpotato around the world, with the majority being foliar feeders, followed by stem, vine, root, and flower feeders (Chalfant et. al., 1990). In the southeastern United States, sweetpotato growers identify cucumber beetles, sweetpotato weevils, white grubs, and sweetpotato flea beetles as causing the most economic damage to their crop (Johnson, 2015). Larval stages of these pests feed directly on the storage roots causing losses in yield and marketability (Ames et al., 1996). These pest problems often result in reduced income for the grower (Nwosisi et al., 2021).

Across the globe, sweetpotato weevils (*Cylas formicarius*) (Coleoptera: Curculionidae) are considered the most important insect pest of sweetpotatoes in the field or in storage (Chalfant et. al., 1990). *Cylas formicarius elegantus* is found throughout the southern United States, from Texas to coastal North Carolina (Chalfant et. al., 1990). However, in North Carolina the weevil only attacks the wild *Ipomoea* spp. found on the Outer Banks (Sorensen, 1987). Weevil feeding induces terpenoid and phenol production in the plant which results in bitter, unpalatable sweetpotato roots (Sorensen, 2009). Because of this, even low sweetpotato weevil populations can cause extensive economic damage. In fact, over $7 million of crop loss in the southern United States is due to sweetpotato weevil (Sorensen, 2009). Sweetpotato weevils complete their

life cycle in approximately 33 days at temperatures between 27 and 33°C (Sherman and Tamashiro, 1954). Females deposit individual cream-colored eggs into natural cavities created in sweetpotato vines and fleshy roots before sealing each cavity with a fecal plug (Chalfant, 1990). Eggs hatch into legless, white first instar larve after approximately 8 days (Sherman and Tamashiro, 1954). Larvae feed by tunneling throughout the sweetpotato root, molting through three instars before pupating (Sherman and Tamashiro, 1954). Mature larvae pupate for 7-10 days in the sweetpotato root or stem, and the adults emerge by chewing through the plant tissue (Capinera, 2018). Adults are 5.5-8mm long, with a black head and abdomen, and reddish brown thorax and legs. The long rostrum with antennae attached at the midpoint is the sweetpotato weevil's most striking feature (Capinera, 2018). Adult females feed for a day or more before mating, and lay eggs soon afterwards (Capinera, 2018). In the United States, strict quarantines have been imposed to limit the spread of the sweetpotato weevil. Restrictions on the shipment of sweetpotato vines and fleshy roots exist at ports of entry and in 14 states (Sorensen, 2009). There are additional restrictions in fourteen southern Alabama counties, which are included in the sweetpotato weevil quarantine area (Harden, 2015). Sweetpotatoes entering Alabama from other states must be authorized by their state of origin to be appropriately inspected and found to be apparently pest free (Harden, 2015). If the sweetpotatoes originated from an area infested with sweetpotato weevil, the products must be properly fumigated to eliminate weevils (Harden, 2015).

Sweetpotato roots are attacked by a complex of insect pests, including wireworms (*Conoderus* spp., *Melanotus* spp., and *Heteroderes* spp.) (Coleoptera: Elateridae), cucumber beetles (*Diabrotica* spp.) (Coleoptera: Chrysomelidae), flea beetles (Systena spp.) (Coleoptera: Chrysomelidae), sweetpotato flea beetle (*Chaetocnema confinis* (Crotch)) (Coleoptera:

Chrysomelidae), and white grubs (*Plectris aliena* (Chapin) and *Phyllophaga ephilida* (Say)) (Coleoptera: Scarabaeidae) (Schalk et al, 1993). This group of pests is known as the Wireworm-*Diabrotica-Systena* or "WDS complex" because the larvae of these beetles cause damage to sweetpotato roots that cannot be differentiated at harvest (Schalk et al, 1993). WDS complex damage consists of shallow feeding holes ranging from less than 1mm to 8 mm in diameter (Schalk et al, 1993). However, feeding from these larvae can sometimes reach the vascular cambium, and their holes deepen as the sweetpotato root grows (Schalk et al, 1986).

Wireworms are the larvae of click beetles (Coleoptera: Elateridae) (Vernon and van Herk, 2022). They are significant pests of many crops, especially potatoes, sweetpotatoes, and corn (Parker and Howard, 2001) (Hermann et al., 2013). Wireworm species complexes vary with location, but several species cause economic damage to sweetpotatoes in the Southeastern United States (Sorensen, 2009). These include the southern potato wireworm *Conoderus falli*, the tobacco wireworm *C. vespertinus*, and the gulf coast wireworm *C. amplicollis. Conoderus scissus* and *C. rudis* were found to be the predominant wireworm species in Georgia (Seal et al, 1992). The corn wireworm *Melanotus communis* (Gyllenhal) is destructive to sweetpotato along the east coast of the United States (Sorensen, 2009). Wireworm feeding reduces sweetpotato quality and results in small holes, narrow tunnels, and scarring to the periderm (Johnson et al., 2008). Wireworms' life cycles vary from 2-3 months for *C. rudis* and *C. falli* to 2-3 years for *C. scissus* and *C. amplicollis* (Seal et al., 1992). Most wireworms spend their immature life stages underground, and many species overwinter as larvae in the soil (Willis et al., 2010). Adult click beetles oviposit on the soil near the crop but do not feed on it (Sorensen, 2009). Sweetpotatoes are at risk of wireworm damage from the beginning of storage root development in early summer

until harvest in the fall (Chalfant et al., 1992; Willis et al, 2010). Thus, few sweetpotato crops are produced entirely free from wireworm damage (Cuthbert, 1965).

The banded cucumber beetle (*Diabrotica balteata)* and spotted cucumber beetle *(D. undecimpunctada howardi)* are also members of the WDS complex. *Diabrotica balteata* is characterized by alternating green and yellow bands on their elytra, while the elytra of *D. undecimpunctada howardi* have 11 black spots on a yellow-green background (Sorensen, 2009). Larvae of this genus chew small holes through the periderm of sweetpotato roots which enlarge as the roots develop (Sorensen, 2009). Feeding holes are often found in groups and occur during early root development, resulting in unattractive scarring upon harvest (Sorensen, 2009). Adults lay eggs in the soil which hatch in 1-2 weeks, depending on temperature. The larvae of these species are almost indistinguishable from each other, and this stage lasts for 8-30 days but varies with food availability. Pupae are formed in cells just beneath the soil surface and adults emerge after one week. In the warm climate of the Southeastern United States, these insects can overwinter as adults which feed on plants in the Convolvulaceae family (Sorensen, 2009).

Three *Systena* species, the elongate flea beetle (*Systena elongata*), the pale-striped flea beetle (*Systena blanda*), and the red-headed flea beetle (*Systena frontalis*) feed on developing sweetpotato roots (Sorensen, 2009). The larvae of these species produce root damage that includes small holes and winding tunnels under the surface of the periderm (Sorensen, 2009). Pinhole sized holes in the root surface are caused by late-season flea beetle feeding (Sorenson, 2009). *Systena* species, specifically *Systena frontalis*, undergo complete metamorphosis, and their eggs are white, oval-shaped, and approximately 1mm long (Herrick and Cloyd, 2020). The larvae are 5-10mm long with creamy white bodies and brown head capsules. Larvae grow through three instar stages in the soil where they feed on plant roots before pupating (Herrick and

Cloyd, 2020). After pupating, adults emerge and are 5mm long with shiny black bodies and red heads (Herrick and Cloyd, 2020). Adults possess enlarged hind femurs, which allow them to jump like fleas, hence their common name “flea beetle” (Cloyd and Herrick, 2020).

Caterpillars like beet armyworm, corn earworm, and soybean looper can cause defoliation damage late in the sweetpotato season (Jennings et. al., 2019). However, infestation by these pests typically occurs after root bulking, so they cause minimal economic damage. Economic damage is possible if caterpillars are present at high levels in the late season and feed directly on exposed sweetpotato roots.

Chemical management

Pre-plant management of *M. incognita* is essential to prepare for a successful growing season. Nematode management generally includes three main strategies: nematicides, cultivar selection, and cultural practices (Overstreet, 2013). In general, there are two types of nematicides: fumigant and non-fumigant (Liu and Grabau, 2022). Fumigant nematicides are highly effective broad-spectrum products that move through the soil as a gas but are highly toxic and can be hazardous for human health (Desaeger, 2020). Because of this danger, fumigant nematicides are facing increasing regulatory pressure, including a ban on several widely used fumigants like methyl bromide (Desaeger, 2020). Fumigant nematicides authorized for use in sweetpotato production include Telone II (1,3-Dichloropropene) Dow AgroSciences, Indianapolis, IN; Vapam HL (Sodium methyldithiocarbamate) AMVAC, Newport Beach, CA; Dominus (Allyl isothiocyanate) Gowan Company LLC, Yuma, AZ; Pic-Clor 60 (1,3 dichoropropene and chloropicrin) TriCal, Inc, Hollister, CA; and K-Pam HL (Potassium N-methyldithiocarbamate) AMVAC, Newport Beach, CA (Grabau and Noling, 2021). Non-fumigant nematicides are applied in liquid or granular formulations and move through the soil as

a liquid. In general, these nematicides can be applied several times per year (Desaeger et al., 2020). The non-fumigant chemical nematicides Vydate L (oxamyl) Corteva AgriScience, Wilmington, DE; Velum (fluopyram) Bayer CropScience, Monheim, Germany; Nimitz (fluensulfone) Adama, Ashdod City, Israel; Mocap EC (ethoprop) AMVAC, Newport Beach, CA; and Mocap 15G (ethoprop) AMVAC, Newport Beach, CA; are labelled for control of *M. incognita* in sweetpotato (Grabau and Noling, 2021). The nematicide AgLogic 15G (aldicarb) AgLogic Chemical LLC, Chapel Hill, NC, is restricted for use in sweetpotato and only authorized in Louisiana and Mississippi at this time (Webb, 2017).

Chemical nematicides and soil fumigants are often used to manage plant-parasitic nematodes like *M. incognita*, but these practices are prohibited in organic systems (Greene and Kremen, 2003). Thus, organic growers are utilizing biological control products to manage *M. incognita* in sweetpotato. Biological nematicides available to sweetpotato growers include Majestene (heat killed *Burkholderia rinojensis* strain A396 cells and spent fermentation media) ProFarm Group, Davis, CA, and MeloCon WG (*Purpureocillium lilacinum* strain 251) Certis Biologicals, Columbia, MD (Grabau and Noling, 2021).

Soil applied insecticides are commonly used to manage soil insect populations (Chalfant et al., 1990). The New England Vegetable Management Guide notes that bifenthrin (Brigade 2EC) FMC Corporation, Philadelphia, PA; can be used for the management of wireworms and white grubs (Wallingford, 2023). Bifenthrin can be applied as a soil-incorporated broadcast, bed, or in-furrow spray at planting, or as a soil directed incorporated spray at cultivation or fertilizer lay-by application. This product can also be applied as a foliar spray to manage click beetles (adult wireworms) and May/June beetles (adult white grubs). Rates vary depending on application method and target species (Wallingford, 2023). Ethoprop (Mocap 15G) AMVAC,

Newport Beach, CA; can be applied and incorporated into the top 2-4" of soil 2-3 weeks before planting to manage wireworms and white grubs (Wallingford, 2023). Belay (clothianidin) Valent, San Ramon, CA; can be applied at planting and at cultivation to manage wireworms (Coolong et al., 2012). Movento (spirotetramat) Bayer CropScience, Monheim, Germany; is labeled for wireworm management in sweetpotato. This systemic insecticide can be applied to the foliage or by chemigation (Webb, 2017). Imidan 70W (phosmet) Gowan Company, Yuma, AZ; is labelled for the control of sweetpotato weevil, banded cucumber beetle, white grub, and wireworm in sweetpotatoes (Webb, 2017). The pyrethroid Baythroid XL (beta-cyfluthrin) Bayer CropScience, Monheim, Germany; is a restricted use pesticide labelled for the control of cabbage looper, cucumber beetles, flea beetles, and sweetpotato weevil adults (Webb, 2017).

Varietal management

Varietal selection is a very important decision for sweetpotato growers. There are hundreds of sweetpotato varieties which are divided into groups based on color of the skin and flesh (Coolong et. al., 2012). Some commercially available sweetpotato cultivars have greater disease and insect tolerance. In commercially available varieties, nematode resistance is most common to *M. incognita* (Jatala and Bridge, 1990). 'Covington' is a commonly planted sweetpotato variety developed at North Carolina State University that is resistant to *M. incognita* race 3 and yields similarly to standard susceptible variety 'Beauregard' (Yencho, et al., 2008). 'Bonita' and 'Evangeline' were developed by researchers at the Louisiana Agricultural Experiment Station and are both rated as highly resistant to *M. incognita* race 3 (La Bonte et al, 2011). 'Hernandez' and 'Jewel' have been rated as moderately resistant to *M. incognita* race 3 (La Bonte et al., 1992). Although less common, some sweetpotato varieties carry resistance to key insect pests. The varieties Murasaki-29 and NC04-531, a clone developed at North Carolina

State University, are rated as moderately resistant to WDS complex and flea beetle damage (Jennings et al., 2019). The sweetpotato cultivars ‘Excel,’ ‘Regal,’ ‘Resisto,’ and ‘Southern Delite’ are considered resistant to WDS complex damage, and ‘Jewel’ and ‘Centennial’ were intermediate (Schalk et al., 1993).

Cultural management

Crop rotation with a non-host of *M. incognita* is effective in reducing nematode populations, however crop selection can be difficult due to its broad host range (Abad et al., 2003). Peanut is a non-host of *M. incognita,* indicating that it is a good crop rotation partner with sweetpotato to reduce *M. incognita* populations in the Southeast (Davis and Webster, 2005). Other crops like cabbage, mustard, and radishes are moderately resistant to *M. incognita*, making them suitable to rotation for nematode management (Bilgrami and Khan, 2022).

Since wireworms have long and varied life cycles, long-term crop rotation away from hosts of wireworm is an effective method to mitigate root damage (Jennings et al., 2019). Growers should avoid rotating with corn and small grains since wireworm species preferentially oviposit in these crop,s and instead should consider planting soybean which is a less desirable host plant (Jennings et al., 2019). Additionally, weedy fallow fields can be a risk factor for wireworm damage, as weeds can be important alternate hosts for wireworm larvae. Winter weed management can reduce the risk of root damage due to overwintering larvae in subsequent sweetpotato planting (Jennings et al., 2019). Also, growers should avoid planting sweetpotato in fields that have been recently converted from pasture, since grasses are preferred hosts of several economically important wireworm species. The interval between pasture conversion and planting of a sensitive root crop like sweetpotato should be several years to minimize the risk of damage.

Biological control

Biological control is the reduction of pest populations by natural enemies like predators, parasitoids, and pathogens (Xiang et al., 2017). *Beauveria bassiana* (Bals.) Vuill. is a naturally occurring soil fungus that causes white muscardine disease upon contact with insect hosts (Groden, 2012). This biocontrol agent is environmentally safe and poses zero or minimal threats to human health (Mascarin and Jaronski, 2016). It has been formulated into several products including BotaniGard 22WP Certis Biologicals, Columbia, MD; Mycotrol Certis Biologicals, Columbia, MD; and Naturalis-L Fargro, West Sussex, England (Groden, 2012). When applying these products, a high spray volume is recommended so that plants are wetted thoroughly, but the product does not run off the leaves (Groden, 2012). The spores of *Beauveria bassiana* are inactivated by sunlight, so prolonged activity can be gained by using drop nozzles or other equipment that can reach the underside of the leaves (Groden, 2012).

Entomopathogenic nematodes can also be effective in the management of sweetpotato insect pests. These species of nematodes often belong to the *Steinernematid* and *Heterorhabditid* families and are most effective against insects in cryptic and soil habitats (Kaya and Gaugler, 1993). The infective juvenile stage enters a host insect through its natural openings (spiracles, mouth, or anus) and penetrates the insect's hemocoel (Kaya and Gaugler, 1993). Entomopathogenic nematodes carry *Xenorhabdus* bacteria in their intestinal tracts, and upon penetration of the hemocoel, release these bacteria into the insect's hemolymph (Kaya and Gaugler, 1993). *Xenorhabdus* bacteria populations multiply quickly within the insect and typically kill the host within 48 hours (Kaya and Gaugler, 1993). The nematodes remain inside the insect cadaver, feeding on its tissue and producing 2 to 3 generations before the next generation of infective juveniles emerge to find new hosts (Kaya and Gaugler, 1993). When it

comes to sweetpotato insect pests, the entomopathogenic nematode species *Steinernema feltiae*, *Steinernema carpocapsae*, and *Heterorhabditis bacteriophora* were shown to significantly increase sweetpotato weevil mortality in-vitro when compared with a water control (Mannion and Jansson, 1992). Additionally, a field trial performed by Schalk, Bohac, and Dukes (1993) found that the entomopathogenic nematode *Steinernema carpocapsae* significantly reduced damage on sweetpotato roots from wireworms, *Diabrotica* spp., *Systena* spp., and sweetpotato flea beetle when applied three times at monthly intervals (Schalk et al., 1993).

Several OMRI-approved biological insecticide options that have activity on wireworms are available (Jennings et al., 2019). These insecticides are typically applied and incorporated into the soil before the formation of beds and repeated when applying a layby application of fertilizer (Jennings et al., 2019). Thorough soil incorporation is critical to create an insecticidal barrier that will restrict the movement of insects into the root zone. For best results, it is recommended to use the highest labeled rate at a high spray volume to ensure the product is applied uniformly across the soil (Jennings et al., 2019).

Caterpillars can be managed by using OMRI approved insecticides with Spinosad and *Bacillus thuringiensis* (*Bt*) active ingredients (Jennings et al., 2019). Using the highest labelled rate, increasing spray volume, and using a spreader-sticker adjuvant can improve canopy coverage. This is essential because the caterpillars must feed on treated leaves for the products to be effective (Jennings et al., 2019). Follow up applications may be necessary to manage these pests when their populations are high. The organic insecticide azadirachtin can effectively manage defoliating pests. This compound is an insect growth regulator and antifeedant but does not kill adult insects (Webb, 2017).

Cover Crops

Cover crops are defined by the Sustainable Agriculture Research and Education program as “a plant that is used primarily to slow erosion, improve soil health, enhance water availability, smother weeds, help control pests and diseases, increase biodiversity, and bring a host of other benefits to your farm” (Clark, 2015). These crops are grown with the intention of incorporating their residue into the soil and are not sold (Fageria et al, 2005). Both grasses and legumes are grown as cover crops, but they affect the system differently. Leguminous cover crops can reduce the need for nitrogen fertilization in the upcoming cash crop due to their ability to biologically fix nitrogen (Fageria et al, 2005). Grass cover crops are often used as tools to reduce erosion and NO_3 leaching (Fageria et al, 2005). In the Southeastern United States, winter cover crops are established after harvest of the preceding cash crop, typically in late summer or early fall (Timper et al, 2006). The cover crops in this region are living and growing through the winter months and are terminated in the spring by mowing, rolling/crimping, or herbicide application (Timper et al., 2021). Cover crops can be used to suppress plant-parasitic nematode populations either by their non-host status, producing allelopathic compounds, and/or enhancing nematode antagonists present in the soil (Wang et al, 2006). Non-host winter cover crops minimize the reproduction of plant-parasitic nematodes over warm winters, reducing nematode populations for the subsequent cash crop season (Timper et al, 2006). However, plant-parasitic nematode host status is variable depending on the variety of the cover crop and the nematode present in the specific field. Some hosts and varieties support higher levels of nematode reproduction than others (Timper et al, 2006). For instance, rye was found to be a relatively poor host of *M. incognita* when compared with crimson clover and hairy vetch in a greenhouse evaluation (Timper et al., 2021). Rye produces benzoxazinoids, secondary metabolites that are toxic to plants, microorganisms, insects, and nematodes (Timper et al., 2021). Rye was also found to

decrease *M. incognita* reproduction and reduce root galling on the following cash crop (Timper et al., 2006). Radish has been classified as a poor host of *M. incognita* with a reproductive factor of 0.9 and low galling index, which indicates that it could be a suitable winter cover crop for *M. incognita* management (Anwar and McKenry, 2010). Additionally, mustard has potential as a winter cover crop for *M. incognita* management due to its low reproductive factor of 0.7 and low galling index (Anwar and McKenry, 2010). Seed meals produced from mustard have been found to suppress plant-parasitic nematodes, including those in the *Meloidogyne* genus (Meyer et al., 2011). The mechanism is thought to be toxin production resulting from the breakdown of glucosinolates contained in mustard plant tissue (Meyer et al., 2011). This shows that mustard shows promise as a *M. incognita* suppressive winter cover crop.

Winter cover crops can also impact soil insect populations. A chief concern of growers when considering adding winter cover crops to their operations is the possibility of a "green bridge effect" (Pellegrino et al., 2021). In fact, despite the benefits of winter cover cropping, adoption of this practice is low in vegetable production systems in the Southeastern U.S. (O'Connell et al., 2015). The green bridge effect occurs when plants are established during a time that the land is typically fallow, allowing a larger population of soil insect pests to survive the winter season (Favetti et al., 2017). Winter cover crops could provide food and microhabitats that promote pest survival and increase the risk of insect damage to the upcoming cash crop (Favetti et al., 2017). This risk is of particular concern in sweetpotato, since it is a root crop, and its primary insect pests are soil-borne. Thus, it is of upmost importance that winter cover crops be assessed for their effects on insect pest damage to the following sweetpotato crop.

Free living nematodes

Soil nematodes can be used as bioindicators to evaluate the effects of agricultural management practices on soil health and the soil food web (Wang, et. al, 2006). The soil food web is key to supporting both soil and plant heath, but agricultural management practices can disturb the soil food web (Wang et al., 2011). Healthy soil food webs support nematodes with differing feeding behaviors and life strategies (Bongers and Bongers, 1998). Nematologists use the Maturity Index (MI), enrichment index (EI), channel index (CI), and structure index (SI) to monitor soil health and describe the soil food web (Paudel et. al., 2021).

The MI is the weighted mean colonizer-persister (cp) value of all the nematodes in a sample, excluding plant parasites and dauerlarvae, which are a specialized larval stage in which development is paused whose existence is triggered by environmental stress (Karp, 2018). The MI is given a numerical range from 1 (often encountered after soil fertilization) to 4 (undisturbed environments) based on the colonizer-persister nematode classification (Bongers and Bongers, 1998). This ranges from colonizers which have a short lifespan but high reproduction rate, to persisters that have a long lifespan and low reproduction rate (Bongers and Bongers, 1998). Colonizers generally have smaller body sizes than persisters, when comparing species at the order level (Bongers and Bongers, 1998). The colonizer-persister (cp) scale consists of five classifications: cp1- cp5 (Bongers and Bongers, 1998). When nutrients are plentiful, cp-1 nematodes dominate. Under heavy metal toxicity, cp-2 nematodes fare best (Bongers and Bongers, 1998). When members of groups cp-3, 4, and 5 are present, it is indicative of low stress and advanced succession (Bongers and Bongers, 1998). Nematodes classified as cp-1 are colonizers with a short generation time and are known for their production of many small eggs. They have high metabolic activity and are only active when soil microbial activity is high. They exhibit exponential population growth under food-rich conditions. Cp-1 nematodes are

characterized by their ability to form dauerlarvae when microbial activity is low, and food is less abundant. Members of the cp-1 classification include the bacterial feeding nematode families of *Rhabditidae*, *Diplogasteridae*, and *Panagrolaimidae* (Bongers and Bongers, 1998). Cp-2 nematodes also have a short generation time and high reproduction rate, but do not form dauerlarvae. They are very tolerant to pollutants and soil disturbance and are found in both food-rich and food-poor conditions. Cp-2 nematodes include *Anguinidae*, *Aphelenchidae*, and *Aphelenchoididae*. Cp-3 nematodes have longer life cycles and are somewhat sensitive to disturbances. Cp-3 nematodes include *Araeolaimida*, *Chromadorida*, and *Diphtherophorida* (Bongers and Bongers, 1998). Cp-4 nematodes have a long generation time and a permeable cuticle that makes them sensitive to pollutants. Excluding predatory nematodes, members of this group are relatively immobile. Cp-4 is comprised of small *Dorylaimids*, *Alaimidae*, and *Bathyodontidae*. Cp-5 nematodes are characterized by their long-life cycles and low reproduction rates. These characteristics are indicative of low metabolic activity. They are very sensitive to soil disturbances and pollutants due to their permeable cuticles. This group is made up of the larger *Dorylaimids*, including predators, omnivores, and plant parasites.

Since nematodes are abundant and respond rapidly to changes in resource availability, their populations can be used to observe changes from land management practices (Du Preez et al., 2022). These ecological indices, like maturity, enrichment, channel, and structure indices, that are based on nematode feeding groups are useful tools to monitor soil health through soil nematode populations (Bongers and Bongers, 1998). These indices allow scientists to describe changes in the soil food web due to nutrient status, soil fertility, and the effects of soil contaminants (Bongers and Ferris, 1999). To perform these analyses, identifying nematodes to the genus or family level is efficient and suitable, depending on the taxon (Bongers and Bongers,

1998). Analyzing nematode abundance based on feeding group allows scientists to describe changes in decomposition pathways and determine the effects of agricultural management practices like fertilization or winter cover cropping on the soil food web (Du Preez et al., 2022).

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Chapter 2: Evaluation of winter cover crops and biological control products to manage *Meloidogyne incognita* and insect pest damage in organic sweetpotatoes

Introduction

Sweetpotato (*Ipomoea batatas*) is a globally significant crop, ranking as the seventh most important food crop worldwide (FAO, 2020). Cultivated primarily for its starchy, nutrient-dense roots, sweetpotatoes are used for various purposes, including human consumption, animal feed, and industrial applications such as ethanol and biofuel production (Bovell-Benjamin, 2010). In the United States, the Southeast is the major sweetpotato-producing region, with the primary production states being Alabama, Louisiana, Mississippi, and North Carolina (USDA, 2023). The vegetable's adaptability to tropical and subtropical regions, coupled with its drought tolerance and ability to thrive in low-fertility soils, makes sweetpotato well-suited for low-input production and making it feasible to be produced organically (Mukhopadhyay, et al., 2011). Organic agriculture has increased in popularity, and there has been a rise in consumer demand for organic products, including fruits and vegetables (Lotter, 2003). Vegetables, which includes sweetpotatoes, are cultivated on 57% of American organic farms (Greene and Kremen, 2003). Organic farming relies on ecologically based pest and fertility management strategies and avoids synthetic pesticides (Greene and Kremen, 2003). The perceived benefits of the organic production model include improved soil health, reduced pesticide usage, ecological harmony, and lower energy input (Lotter, 2003).

Plant-parasitic nematodes and insect pests pose a significant threat to sweetpotato crops, and in the Southeast, the southern root-knot nematode (*Meloidogyne incognita* (Kofoid and White)) is a particularly damaging species (Kim and Yang, 2019). *Meloidogyne incognita*

infections result in root galling, reduced plant vigor, and lower yields (Bird, 1974). In conventional production, both fumigant and non-fumigant chemical nematicides are utilized, but these practices are not aligned with organic farming (Liu and Grabau, 2022). Consequently, organic growers turn to biopesticides, such as Majestene and MeloCon WG, and cultural practices, like winter cover cropping, to manage nematode infestations (Greene and Kremen, 2003; Timper et al., 2006).

Insects, including sweetpotato weevils (*Cylas formicarius* Fabricius), white grubs (*Phyllophaga* spp.), wireworms (*Condoderus* spp., *Melanotus* spp., and *Heteroderes* spp.), cucumber beetles (*Diabrotica balteata* LeConte and *D. undecimpunctata howardi* Barber), and sweetpotato flea beetles (*Chaetocnema* spp.), also contribute to economic losses for sweetpotato growers (Ames et al., 1996). Chemical insecticides, such as bifenthrin and Imidan 70W, are commonly used in conventional farming for managing insect pest populations, however organic growers must rely on biological control methods, employing the entomopathogenic fungus *Beauveria bassiana* (Bals.-Criv) Vuill. and entomopathogenic nematodes like *Steinernema feltiae* (Filipjev, 1934), *Steinernema carpocapsae* (Weiser, 1955), and *Heterorhabditis bacteriophora* (Poinar, 1975) to manage insect pest damage on their farms (Webb, 2017; Groden, 2012; Kaya and Gaugler, 1993). Cultural practices, such as crop rotation with non-hosts like peanuts for nematode management and soybeans for wireworm management, contribute to sustainable sweetpotato cultivation (Jennings et al., 2019; Davis and Webster, 2005). Additionally, cover crops, both grasses and legumes, are employed to improve soil health, reduce erosion, and control pests (Clark, 2015).

In summary, sweetpotato is an economically important crop in the Southeastern U.S. with the potential to be produced organically. However, sweetpotato faces substantial pest pressure in

this region from insects like those that make up the WDS pest complex (wireworm spp., *Diabrotica* spp., and *Systena* spp.) and plant-parasitic nematodes like *M. incognita*. Therefore, it is critical to develop effective organic integrated pest management practices for growers. The objectives of this work were (1) to evaluate biopesticides for the management of *M. incognita* and insect pests, (2) to determine the efficacy of winter cover crops in the suppression of *M. incognita* populations and insect pest damage, and (3) to assess the impact of winter cover crops on soil health indicators.

Materials and Methods

Nematode inoculum

Meloidogyne incognita race 3 used for inoculum in these experiments were increased on corn maintained in 500 cm^3 polystyrene pots in the greenhouse at the Plant Science Research Center in Auburn, AL. All soil used to increase nematodes and in the greenhouse trials was a Kalmia loamy sand textured soil (80% sand, 10% silt, 10% clay, 1.2% organic matter, pH 6.9) sourced from Auburn University's Plant Breeding Unit (Tallassee, AL). The soil was pasteurized at 88°C for 12 hours, allowed to cool for 24 hours and pasteurization was repeated. The pasteurized soil was combined with sand at a rate of 1:2 soil to sand. Fertilizer and lime were added at rates recommended by Auburn University Soil Testing Laboratory. Nematode eggs were extracted from the roots of approximately 60-day-old corn stock cultures. Corn shoots were discarded, and the roots were gently washed in water to remove excess soil. Nematode eggs were extracted through a modified method by Hussey and Barker by placing the roots in a 0.625% NaOCl solution and shaking at 1 G force for four minutes on a Barnsted Lab Line Max Q 5000E Class shaker (Thermo Fisher Scientific: Waltham, MA) (Hussey and Barker, 1973). The roots were gently scrubbed under running water, and dislodged eggs were collected on a 25 µm pore sieve. Using a method modified by Jenkins (1964), the egg solution was transferred into 50 mL centrifuge tubes, processed by sucrose centrifugation-flotation in 1.14 specific gravity, and centrifuged at 1400 rpm for one minute. The eggs in the supernatant of the sucrose solution were collected on a 25 µm pore sieve and rinsed well with water. *Meloidogyne incognita* egg density was determined by enumerating the eggs using a Nikon TSX 100 inverted microscope at 40X magnification. Egg density was adjusted to 5,000 eggs/mL.

Greenhouse tests

Biopesticides: Trials to evaluate the impacts of biopesticides and winter cover crops on *M. incognita* race 3 were conducted initially in the greenhouse. Ten biopesticides AzaGuard; Azadirachtin (BioSafe Systems, LLC, Hartford, CT) BotaniGard 22 WP; *Beauveria bassiana* strain GHA (Certis Biologicals, Columbia, MD), BoteGHA ES; *Beauveria bassiana* strain GHA (LAM International Corporation, Butte, MT), Chitocide; (Concept AgriTek, Charleston, MO), Majestene; heat-killed *Burkholderia* spp. strain A396 cells and spent fermentation media (ProFarm Group, Davis, CA), MeloCon; *Paecilomyces lilacinus* strain 251 (Certis Biologicals, Columbia, MD), Minuet; *Bacillus subtilis* strain QST 713 (Bayer CropScience, St. Louis, MO), Monterey Nematode Control; Saponins of *Quillaja saponaria* (Lawn and Garden Products, Inc., Fresno, CA), Promax; Thyme oil (Bio Huma Netics, Inc., Gilbert, AZ), and Seduce; Spinosad (Certis Biologicals, Columbia, MD), plus the conventional product Velum Prime; Fluopyram (Bayer CropScience, St. Louis, MO) were compared to an untreated control. One 15cm 'Beauregard' variety sweetpotato slip was planted per 500 cm^3 polystyrene cup (Dart Container Corporation, Mason, Michigan) filled with the pasteurized soil mixture. At planting, 5,000 *M. incognita* eggs were pipetted to each pot in a 2.5-cm depression in 1 mL of water and covered with soil to prevent desiccation. After nematode inoculation, the biopesticide treatments were applied at labeled rates (Table 1) via 50mL drench treatments. The biopesticide treatments were applied 30 days after planting for the second application.

Winter cover crops: To determine the reproduction rate of *M. incognita* race 3 on winter cover crops, seven individual winter cover crops and two winter cover crop mixes were selected. The winter cover crops tested included *Avena strigose* L.; black oats, *Trifolium incarnatum* L.*;* crimson clover, *Raphanus sativus* var. longipinnatus L.; daikon radish, *Secale cereale* L.*;* elbon

rye, *Pisum sativum* L.*;* field pea, *Triticum aestivum* L.*;* wheat, and *Sinapis alba* L.*;* yellow mustard. Winter cover crop mix 1 consisted of crimson clover, field pea, yellow mustard, black oat, daikon radish, and elbon rye, while winter cover crop mix 2 consisted of crimson clover, daikon radish, elbon rye, and wheat. These cover crops were compared to a fallow- unplanted control. Seeds were obtained from Piedmont Fertilizer Company (Opelika, AL) and planted at recommended rates into 500-cm^3 polystyrene cups. The winter cover crop test was similarly inoculated with 5,000 *M. incognita* eggs pipetted to each pot at two weeks after cover crop planting when the cover crop seedlings had emerged.

Experimental design: The biopesticides and winter cover crops in each set of tests were arranged in a randomized complete block design (RCBD) with five replications, and each experiment was repeated. Plants were hand watered as needed to maintain soil moisture. Greenhouse temperatures ranged from 25°C to 29°C over the course of this test. Lighting was provided by 1000-watt halide bulbs which produced 110,000 lumens for a 14-hour day length.

Sweetpotato roots in the biopesticide tests were harvested ≈45 days after planting (DAP) and the cover crop tests were harvested at 74 DAP. Plant measurements included root fresh weight (RFW), shoot fresh weight (SFW), and biomass (RFW + SFW). *Meloidogyne incognita* population density was also recorded by extracting the total number of eggs from each root system and was reported as eggs per gram of root. Nematodes were extracted from the roots using a combination of gravity sieving and sucrose centrifugal flotation (previously described) and enumerated with a Nikon TSX 100 inverted microscope at 40X magnification.

Microplot tests

Biopesticides and winter cover crops were tested to determine suppression of *M. incognita* in microplot experiments established at the Plant Science Research Center (PSRC) in Auburn, AL, and each test was repeated. Microplots consisted of a pot within a pot design with a 23-liter plastic tree pot (Grip Lip 2800; Nursery Supplies Inc., Montgomery, AL) nested inside an identical 23-L plastic pot with a brick placed between to serve as a root barrier and then set into the ground. Microplots were filled with a Kalmia loamy soil (fine-loamy over sandy or sandy skeletal, siliceous, semiactive, thermic Typic Hapludults) comprised of 80% sand, 10% silt, and 10% clay mixed as 2 parts field soil with 1 part sand. Water was provided through a drip irrigation system and was adjusted throughout the season as needed. Each microplot was inoculated 250 cm^3 of soil which contained an average of 50,000 eggs and J2 life stages of *M. incognita* race 3 maintained as described in the "Nematode Inoculum" section. The biopesticides test took place from June 2022 until October 2022, and upon its conclusion, the winter cover crops test was initiated. All tests were arranged in a RCBD with 5 replications and each test was repeated. Biopesticides tested were identical to those used in the greenhouse test. For this test, one 15cm 'Beauregard' variety sweetpotato slip was planted per microplot on 3 June 2022, and all nematicide treatments were applied at labelled rates via a 0.5 L drench treatment at planting and again at 35 DAP (8 July 2022). Soil samples were taken 14 September 2022 to determine the efficacy of biopesticides on soil populations of *M. incognita*. The sampling method consisted of collecting four 2.5-cm x 20-cm soil cores at the base of the plant from each microplot. These 4 core samples were combined and mixed to make up a composite 100 cm^3 subsample per pot. Each soil sample was placed in a bucket with approximately 1 liter of water where it was then swirled thoroughly suspend the soil in the water. The mixture was then poured through nested 250 µm-pore and 25 µm-pore sieves, and the contents left on the 25 µm-pore sieve were

collected and washed into 50mL centrifuge tubes (Riggs and Schmitt, 1988). This process was followed by sucrose centrifugation as previously described. Then the nematodes contained in the supernatant of the sucrose solution were collected on a 25 µm-pore sieve and rinsed with water to remove all sucrose. *Meloidogyne incognita* J2 population levels were determined by enumerating the nematodes extracted from the soil using a Nikon TSX 100 inverted microscope at 40X magnification. At plant maturity, ≈130 DAP, microplots were harvested by removing all marketable sweetpotato roots per microplot and their weights were recorded.

Winter cover crop tests: The two winter cover crops tests were planted in a RCBD with 5 replications on 18 November 2022 and grew throughout winter until termination by removing the aboveground biomass and incorporating the roots into the soil in early May 2023. Winter cover crops tested were identical to those included in the greenhouse trial. One 15cm 'Beauregard' variety sweetpotato slip was planted per microplot for the first test on 26 May 2023 and for the second test on 2 June 2023. Soil samples were taken on 18 July 2023 and 25 July 2023 (53 DAP) to determine the effect of winter cover crops on soil populations of *M. incognita*. Soil samples were extracted to measure nematode populations and enumerated as previously described. Microplots were harvested at plant maturity on 19 September 2023 and 26 September 2023 (116 DAP).

Field tests

Field trials were established at two locations: Brewton Agricultural Research Unit (BARU) in Brewton, AL with a Benndale fine sandy loam soil with a particle separation of 73% sand, 20% silt, and 7% clay and a farmer field near Dobson, NC with a Colvard sandy clay loam soil consisting of 53% sand, 27% silt, and 20% clay. Both fields were naturally infested with *M. incognita* race 3. Winter cover crops tested in Alabama included koto buckwheat (2022),

crimson clover, field peas, yellow mustard, black oats, daikon radish, elbon rye, and a mixture of crimson clover, field peas, yellow mustard, black oats, and daikon radish (2023). Winter cover crops tested in North Carolina included crimson clover, daikon radish, elbon rye, wheat, and a mixture of crimson clover, daikon radish, elbon rye, and wheat. Cover crop selection depended on seed availability in each location. In October of each year, winter cover crops were planted in a RCBD with 4 replications in Alabama and 5 replications in North Carolina. Field plots in Alabama consisted of two rows, 7.6-meters long with a 1-meter row spacing and a 1.5-meter alley between replications. In North Carolina, field plots consisted of two rows, 7.6-meters long with a 1-meter row spacing and a 4.6-meter alley between replications. Winter cover crops were terminated in April of each year using a Bush Hog mower (Bush Hog, Inc., Selma, AL), and ground was prepared for planting by tillage and hill formation. Sweetpotatoes were planted on 2 June 2022 and 6 June 2023 in Alabama and 14 June 2022 and 9 June 2023 in North Carolina. '*Beauregard'* variety sweetpotato slips were planted with 1 slip every 0.3-meters using a sweetpotato transplanter (US Small Farm Equipment Company, South Dakota, USA). In Alabama, beginning at two weeks after transplanting, applications of Triple Threat Beneficial Nematodes; *Steinernema feltiae*, *Steinernema carpocapsae,* and *Heterorhabditis bacteriophora* at 123.5 million infective juveniles of each species per hectare, (Arbico Organics, Oro Valley, AZ) and BotaniGard 22 WP; *Beauveria bassiana* strain GHA at 4.9 kilograms/ hectare (Certis Biologicals, Columbia, MD) were applied to one row of each two-row plot using a handheld sprayer every month throughout the growing season. Majestene; heat-killed *Burkholderia* spp. strain A396 cells and spent fermentation media at 18.7 liters/ hectare was included in the first two applications. In North Carolina, the applications were made monthly at the same rates, with Majestene included in the first two applications. Sweetpotatoes were harvested using a D-10T

potato digger, (US Small Farm Equipment Co, Worland, WY) on 3 October 2022 (123 DAP) and 6 October 2023 (122 DAP) in Alabama and 15 October 2022 (123 DAP) and 14 October 2023 (127 DAP) in North Carolina. All sweetpotatoes were graded by size and classified as jumbo, number one, canner, or cull, and the number and weight of each grade was recorded per plot (Figure 1) (Benedict and Smith, 2009).

Field data collection

Soil samples were collected monthly during the growing season to monitor soil populations of *M. incognita* and free-living nematodes including bacterivores, fungivores, herbivores, omnivores, and predators. Soil CO_2 respiration was also determined. Nematodes were extracted from 100cm^3 of soil as previously described and identified to trophic group according to their morphology (Goodey, 1963) using a Nikon TSX 100 inverted microscope at 40-100X magnification. At planting, 30 DAP, and 84 DAP, soil samples underwent CO_2 respiration measurements using the Solvita CO_2 Burst test (Woods End Labs, Augusta, ME). Soil samples were dried using a food dehydrator (Excalibur Products, Sacramento, CA) and run through an 850 µm-pore sieve to remove rocks and other debris. Then a 30cm^3 subsample of each sample was added to the provided internal beaker and interspersed with 9cm^3 water through a water dispersion screen. A low-CO_2 probe was placed into the moistened soil, taking care not to touch the gel portion and internal beakers were placed inside 475mL Solvita jars. Lids were tightly closed, and the samples were maintained at 20°C for 24 hours, after which the results were determined by inserting each probe into the Solvita Digital Color Reader using the CO_2-Low setting. Soil samples were also subjected to phospholipid fatty acid analysis (PLFA) performed by Regen Ag Lab (Pleasanton, NE).

Post-harvest data collection

Upon harvest, a subsample of number one grade sweetpotatoes from each plot were transported to Auburn University's PSRC for insect damage and internal nematode damage assessments. Insect damage was quantified by counting the incidence of WDS complex (small holes), white grub (large irregularly shaped holes), sweetpotato flea beetle damage (winding tunnels under the periderm) on 5 number one grade sweetpotatoes per plot (Reed, et. al., 2009). The values were then averaged to find the average incidence of each type of damage per number one grade sweetpotato.

Data analysis

Data collected from the winter cover crop and biopesticide greenhouse and microplot trials were analyzed by SAS 9.4 (SAS Institute: Cary, NC) using the PROC GLIMMIX procedure. The analysis of variance was conducted with biopesticides or cover crops as the main factor. Means were separated using Tukey Kramer LS-means test at $P \leq 0.05$. Student panels were produced to determine the normality of the residuals. There were no significant interactions between the two repeats of the winter cover crop greenhouse tests, thus data were pooled into a single data set for a total of ten replications. The tests also found no significant interactions between the two runs of the greenhouse and microplot tests and that data were analyzed as one dataset. Field data were also analyzed using the SAS PROC GLIMMIX procedure. The analysis of variance was conducted with dependent variables including winter cover crop biomass, *M. incognita* populations, free living nematode populations, WDS, white grub, flea beetle, and total insect damage, sweetpotato yields by grade, and soil CO_2 respiration. Fixed effects were winter cover crop or biopesticide application and the random effects included replication, and years. Student panels were produced to determine the normality of the residuals. There were no significant interactions between replications or years, so these were considered random effects.

The Poisson distribution was used for insect damage data. LS-means were compared between the cover crop treatments and biopesticides by Tukey Kramer LS-means test at $P \geq 0.10$. LS-means presented in the tables followed by different letters indicate a significant difference. Economic analysis was performed by determining the value of sweetpotato yields using organic sweetpotato prices obtained from a produce packing house. LS-means of economic values were compared between the cover crop treatments and biopesticides by Tukey Kramer LS-means test at $P \geq 0.10$. Canonical analysis of variance was conducted by Dr. Koon-Hui Wang (Professor, University of Hawai'i at Manoa) using Canoco 5.1 (Microcomputer Power: Ithaca, NY).

Results

Greenhouse testing

Biopesticides: Meloidogyne incognita race 3 nematode population density increased to high levels when grown on sweetpotato over the 45-day greenhouse test (Table 1; Figure 2). Biopesticides did not significantly affect sweetpotato root fresh weight, shoot fresh weight or total biomass with all biopesticides producing similar plant weights. No phytotoxicity was observed on the sweetpotatoes with any of the products applied. Velum was the most efficacious product tested and reduced *M. incognita* eggs per root system by 93% compared to the untreated control. Of the biopesticides, Promax and the combination of BotaniGard 22 WP and Triple Threat Entomopathogenic Nematodes supported significantly higher *M. incognita* eggs per root system than Velum but were not significantly different from the other biopesticides tested. All biopesticides supported similar populations of *M. incognita* eggs per root system and eggs per gram of root. MeloCon supported the lowest number of *M. incognita* eggs per gram of root among biopesticides tested. All the biopesticides maintained *M. incognita* population levels at <3,300 eggs and J2 per gram of root. Velum supported significantly fewer *M. incognita* eggs per gram of root than the other biological control products tested and the untreated control ($P \leq 0.05$).

Winter cover crops: Meloidogyne incognita population densities increased to high levels on the winter cover crops in the greenhouse over the 56-day test (Table 2; Figure 3.). The highest root fresh weight was recorded on cover crop mix 1 (crimson clover, field pea, yellow mustard, black oat, daikon radish, and elbon rye), which was numerically similar to cover crop mix 2

(crimson clover, daikon radish, elbon rye, and wheat), elbon rye, black oat, and wheat ($P \leq 0.05$). Crimson clover, field pea, and yellow mustard had the lowest root fresh weights in this test and were not significantly different from the fallow ($P \leq 0.05$). Shoot fresh weights were 79% lower than the root weights with daikon radish producing the largest plants ($P \leq 0.05$) compared to all other winter cover crops. Biomass varied across winter cover crops with the highest biomass ($P \leq 0.05$) recorded on cover crop mix 1 and 2, elbon rye, black oats, and wheat ($P \leq 0.05$). The majority of winter cover crops evaluated supported low *M. incognita* densities. Elbon rye supported the fewest *M. incognita* eggs per gram of root and was statistically similar to all other cover crops tested, excluding field pea ($P \leq 0.05$). The highest *M. incognita* nematode population density was found on field peas which supported a 98% greater *M. incognita* nematode population per gram of root ($P \leq 0.05$) than the average of all the remining cover crops. Crimson clover also supported a high population of *M. incognita* nematodes with 94% higher population density. Excluding fallow, the lowest *M. incognita* nematode reproductive factors of 1.0 were recorded on daikon radish and cover crop mix 2. This indicates that these winter cover crops were found to be poor winter hosts of *M. incognita* race 3 only allowing the nematode to sustain its population. Mix 1, elbon rye, yellow mustard, and black oats would also be considered poor host supporting minimal nematode reproduction. High reproductive factors of 15.3 and 5.0 were recorded on field pea and crimson clover, respectively. Those two cover crops could increase *M. incognita* populations before the summer crop planting.

Summer cover crops: Of the summer cover crops, piper sudangrass and elbon rye produced the highest root fresh weight ($P \leq 0.05$). The summer covers velvetbean and piper sudangrass supported the largest shoot fresh weights ($P \leq 0.05$), however the shoot weights were 80% lower than the root fresh weights. Biomass (sum of root and shoot fresh weights) which was

influenced by the root weights was greatest ($P \leq 0.05$), for piper sudangrass and elbon rye with elbon rye supporting similar biomass as sunn hemp and velvetbean (Table 2; Figure 3). *Meloidogyne incognita* race 3 reproduction was highest on rye which supported 288 *M. incognita* eggs per gram of root at the conclusion of the test and a reproductive factor of 0.058. Sunn hemp and velvetbean both supported low levels of *M. incognita* reproduction with reproductive factors of 0.005 and 0.004, respectively. Velvetbean supported the lowest *M. incognita* populations of the summer cover crops with only 18 *M. incognita* eggs per gram of root, a 94% decrease compared to elbon rye.

Microplot testing

Biopesticides: All biopesticides tested reduced *M. incognita* population densities on sweetpotatoes below the untreated control in these microplot conditions (Table 3; Figure 4.). The lowest *M. incognita* population density of 765 and 773 J2 nematodes/100cm^3 soil was recorded in the microplots treated with MeloCon ($P \leq 0.05$), and the combination of BotaniGard 22 WP and Triple Threat Entomopathogenic Nematodes, respectively. Ranking the biopesticides found *M. incognita* populations were lowest when sweetpotatoes were treated with MeloCon, the combination of BotaniGard 22 WP and Triple Threat Entomopathogenic Nematodes Chitocide, Seduce, Promax, and Minuet. These biopesticides maintained *M. incognita* population levels at less than 900 J2/100cm^3 soil. Biopesticide treatments had no significant effect on marketable yield in the microplots. Although not statistically significant, ranking the biopesticides for yield found the highest marketable yield of 0.56 kg/plant was recorded on the plants treated with Chitocide followed by BotaniGard 22 WP and Triple Threat Entomopathogenic Nematodes, Velum, AzaGuard, and Majestene.

Winter cover crops: Of the winter cover crops tested in the microplots, field peas, elbon rye, and mix 1 (crimson clover, daikon radish, elbon rye, and wheat) produced the highest ($P \leq 0.05$) aboveground biomass at cover crop termination (Table 4; Figure 5). Yellow mustard and the weedy fallow produced the lowest biomass, which was not significantly different from black oats and crimson clover. Prior to sweetpotato planting, field peas supported the highest soil population of *M. incognita* (35 J2/100cm^3 soil), followed by fallow. Daikon radish, elbon rye, crimson clover, cover crop mix 1, black oats, and yellow mustard all supported lower nematode populations than the field peas. At the July sampling date (60 DAP) when sweetpotatoes were growing following the cover crops, the *M. incognita* population density was statistically equivalent across all covers. This trend continued at the September sampling date, with no difference in *M. incognita* populations on the sweetpotatoes following the winter cover crops. Sweetpotato yields were similar across the winter cover crops as well. Ranking numerically the highest sweetpotato yields were harvested from the plots that had been cultivated with black oat, daikon radish, and elbon rye.

Field testing

Brewton, Alabama, 2022-2023

Alabama nematodes: Field peas produced the highest shoot dry weight of the winter cover crops tested at 8,685 kg/ha at termination in the spring (Table. 5; Figure 6). The Alabama winter cover crop mix and the daikon radish winter cover crops produced shoot dry weights numerically similar to field peas. The lowest cover crop shoot dry weight produced was from the black oat, elbon rye, and yellow mustard cover crops which were statistically similar to the fallow plots. At sweetpotato planting, *M. incognita* race 3 populations were similar across winter cover crops, however, the numerically highest populations were recorded following the field pea

cover crop at 193 *M. incognita* J2/100cm^3 soil (Table 5; Figure 7). Daikon radish was most effective at maintaining low *M. incognita* populations, with only 10 *M. incognita* J2/100cm^3 soil recorded at the beginning of the sweetpotato cropping season. At 30 DAP, *M. incognita* J2 populations were low following all winter cover crops probably due to the migration of J2's into the growing sweetpotato roots. However, the plots following crimson clover supported the highest populations of *M. incognita* J2's. At mid-season (60 DAP), *M. incognita* J2 populations were similar as the sweetpotatoes were growing, ranging from a low of 29 to a high of 87 *M. incognita* J2 / 100cm^3 soil after elbon rye and crimson clover cover crops, respectively. *Meloidogyne incognita* populations were high near harvest, with plots following field peas numerically leading at 424 *M. incognita* J2/ 100cm^3 soil. Near harvest, sampled at 84 DAP, *M. incognita* populations were similar across all sweetpotato plots regardless of the winter cover crop grown. Overall *M. incognita* soil populations had increased 83%, on average across all plots, from planting until harvest.

Alabama insect damage: In Alabama, no interaction was observed between the winter cover crops and the biopesticides applied, thus data are presented separately (Table 6; Figure 9). The most damaging insect pests belonged to the WDS complex (wireworm, *Diabrotica*, and *Systena*). Across winter cover crops, damage by this pest complex was similar, although numerically greatest following daikon radish with 9.7 small holes/sweetpotato. The addition of biopesticides significantly ($P \leq 0.05$) reduced the incidence of WDS complex damage of small holes/ potato by 15%. White grub damage, which is visualized as gouging tunnels across the surface of the sweetpotato, was also present in low density in this test. The white grub damage ranged from 0.3 damage incidences/potato following the crimson clover cover crop to 0.1 damage incidences/potato following yellow mustard. The biopesticide application did not

influence white grub damage due to low pest pressure at the experiment site. Sweetpotato flea beetle damage, which is observed as winding tunnels under the sweetpotato periderm, was also low in this test, and the addition of biopesticides reduced ($P \leq 0.05$) the damage incidence by 14% on average, compared with the untreated. Sweetpotato flea beetle damage was similar across all the cover crops. Interestingly, the highest damage was observed following daikon radish at 2.3 incidences/ potato. *Meloidogyne incognita* damage was measured as incidence of root cracking and was statistically similar across all winter cover crops tested, although numerically highest following field peas which also supported numerically larger populations at sweetpotato planting. The total insect damage was driven by WDS complex damage incidence since it was the primary insect pest complex. Although statistically similar following all winter cover crops, daikon radish resulted in numerically the highest total insect damage and black oats resulted in the lowest. The addition of biopesticides ($P \leq 0.05$) reduced total insect damage by 15%.

Alabama yield: Sweetpotato yield was measured by counts, weights, and quality grade (jumbo, number one, and canner). No significant interactions between the winter cover crops and biopesticide applications were observed for sweetpotato yield, so the data is presented separately. Winter cover crops did not significantly affect the number or weight of jumbo, number ones, or canner grade sweetpotatoes (Table 7; Figure 10). The application of biopesticides did not significantly affect the number or weight of jumbos or number one sweetpotatoes. The number and weight of the canners was increased with the application of the biopesticides. Number ones are the most marketable grade of sweetpotato, and the highest amount of number ones was recorded after the field pea winter cover crop, although not statistically significant. Over 800 more number ones were harvested when biopesticides were applied when compared to the

untreated, which was a 352 kg/ha increase or $116 per hectare. The most number ones by weight were measured following the field pea winter cover crop at 8,799 kg/ha, although not statistically significant. This was a 1,762 kg/ha increase over the fallow. There was a significant gain ($P \leq 0.05$) in the number of canners harvested with the addition of biopesticides, an over 5,000 sweetpotato/ ha increase over the untreated, which corresponded to an over 750 kg/ha increase valued at $250 per hectare. There were no significant differences in number of canners harvested across winter cover crops, but the greatest number was produced following field peas. Numerically, the highest weight of canners was recorded following the yellow mustard winter cover crop, and the lowest canner weight occurred following the Alabama winter cover crop mix. The value of the sweetpotato crops was similar ($P \leq 0.05$) between the untreated and the application of the biopesticides; however, the biopesticides increased the value by $60 per hectare. The cover crops also supported similar sweetpotato yields, although field peas, Alabama mix, and elbon rye produced an increase value of $257, $210, and $174 per hectare, respectively.

Alabama soil health: CO_2 respiration is an indicator of healthy soil biological activity, and it was measured across the summer season. In Alabama, there were no statistical differences in microbial CO_2 respiration across cover crops at sweetpotato planting (Table 8; Figure 11). The highest at-plant respiration was recorded on the Alabama winter cover crop mix, followed by black oats and crimson clover. At 30 DAP, numerically, microbial respiration was still highest on the Alabama winter cover crop mix. Microbial CO_2 respiration was highest on elbon rye, but closely followed by Alabama mix and crimson clover at the end of the season, although not significantly different.

<u>North Carolina, 2022-2023</u>

North Carolina nematodes: In North Carolina, the highest ($P \leq 0.05$) cover crop shoot dry weight was recorded on elbon rye (26,404 kg/ha), followed by the NC winter cover crop mix (Table 9; Figure 7). Wheat, crimson clover, and daikon radish supported similar cover crop shoot dry weights. While winter cover crops were actively growing, soil *M. incognita* J2 populations were numerically greatest on elbon rye and lowest on the NC winter cover crop mix, although all were higher than the soil threshold level of 10 *M. incognita* J2/100 cm^3. At sweetpotato planting, *M. incognita* J2 populations were similar following all winter cover crops, ranging from 23 to 77 *M. incognita* J2/100cm^3 soil (Table 9; Figure 7). Sweetpotatoes that followed wheat and fallow had the lowest soil populations of *M. incognita*. No statistical differences in soil *M. incognita* populations were observed at 30 DAP, and populations were similar following all winter cover crops tested, but had nearly doubled since planting sweetpotato. At midseason (60 DAP), *M. incognita* populations increase an average of 66% across all the previous cover crops at 60 DAP. However, populations were numerically lowest on elbon rye at 24 *M. incognita* J2/100cm^3 soil at the same sampling timing. At harvest, ≈84 DAP, *M. incognita* populations were similar across all sweetpotato plots regardless of the NC winter cover crop the previous season. Overall *M. incognita* soil populations had increased 50 % from planting until harvest.

North Carolina nematode community: After two years of winter cover cropping and organic sweetpotato production, soil nematode communities were monitored and compared with a baseline sample from spring 2022. In 2023, Radish supported an increased abundance of bacterivorous nematodes at cover crop termination (Figure 13). However, throughout the second sweetpotato cropping season, crimson clover winter cover crop plots measured an increased the abundance of bacterivorous nematodes, compared to the fallow. The winter cover crop mix initially increased the abundance of fungal-feeding nematodes, but at mid-season the plots

following crimson clover supported the highest fungivore populations, which was significantly ($P \leq 0.05$) greater than fallow plots. However, the winter cover crop mix plots supported the highest populations of herbivorous (plant-parasitic) nematodes recorded at sweetpotato harvest. Richness, measured as number of nematode genera was significantly higher following crimson clover than the fallow ($P \leq 0.05$). Although not statistically significant, crimson clover was associated with higher abundance of omnivorous and predatory nematodes, resulting in the highest Structural Index (SI) at the end of the 2023 sweetpotato cropping season (Figure 14). Though there were no significant differences in nematode Enrichment Index (EI) and Channel Index (CI) among winter cover crops, elbon rye enhanced fungal decomposition at cover crop termination in 2023 with a higher F/ F+B ratio (Fungivores/ Fungivores +Bacterivores) compared to radish and wheat cover crops.

North Carolina insect damage: The WDS complex was the primary insect pest challenge in North Carolina. Sweetpotatoes following the various cover crops experienced similar non-significant WDS complex damage incidences ranging from 2.43 small holes/ number one grade sweetpotato following crimson clover and 3.53 following the fallow plots (Table 10; Figure 15). The application of biopesticides significantly ($P > 0.001$) reduced WDS complex damage, and on average, biopesticides reduced damage by 40% compared to the untreated. White grub damage was low in this location, with no differences between cover crops or the biopesticide application. Sweetpotato flea beetle damage was also low, following the same pattern as white grubs; no significant differences due to winter cover crops or biopesticide applications. Incidences of root-knot nematode damage recorded as root cracking were low. Total insect damage was driven by the WDS complex since it was the primary insect pest challenge in this test. Numerically, the highest total insect damage incidence occurred in sweetpotatoes following

the fallow, and the lowest total insect damage was recorded following the crimson clover cover crop. The addition of biopesticides significantly reduced total insect damage by 36%.

North Carolina yield: In North Carolina as in Alabama, there were no significant interactions in sweetpotato yield parameters between the cover crops and biological applications, so the data is presented separately. Cover crops did not significantly affect the number or weight of jumbo, number ones, or canner grade sweetpotatoes across winter cover crops (Table 11; Figure 16.). The addition of biopesticides did not affect the number or weights of jumbo, number ones, or canner grade sweetpotatoes. The NC winter cover crop mix produced significantly more canner grade sweetpotatoes than fallow, an over 9,000 sweetpotatoes/hectare increase. In canner weight, elbon rye performed best with 4,555 kg/ha, an over 1,200 kg/ha increase over the fallow. Total marketable yield consisted of the combined weight of jumbos, number ones, and canners. The numerically highest total marketable yield was recorded following the wheat winter cover crop with 20,679 kg/ha, an over 2,000 kg/ha increase over the fallow. There was also a numerical yield increase associated with applying biopesticides with the treated plots yielding over 700 kg/ha over the untreated plots. The value of the sweetpotato crops was similar ($P \leq 0.05$) between the untreated and the application of biopesticides; however, the biopesticides increased the value by $33 per hectare. The cover crops also supported similar sweetpotato yields although wheat and daikon radish produced an increase value of $137 and $71 per hectare, respectively.

North Carolina soil health: In North Carolina, microbial CO_2 respiration values were high at sweetpotato planting. The highest value was recorded on the NC winter cover crop mix, followed by wheat, although no statistical differences were seen across winter cover crops at sweetpotato planting (Table 12; Figure 11). At 30 DAP, the highest microbial CO_2 occurred following crimson clover and the NC winter cover crop mix with values of 96.5 and 95.3 ppm,

respectively which was 27% higher than the lowest microbial CO_2 occurring following the Elbon rye. No statistical differences were measured between the different cover crops at 84 DAP, but the highest values of 95.2 ppm were recorded following the wheat and the NC winter cover crop mix. Based on the canonical analysis of variance, sweetpotato yield was positively related to nematode structural index, omnivorous nematode abundance, nematode diversity index, and the ratio of gram-positive to gram-negative bacteria (Figure 12). Higher total microbial biomass measured as total phospholipid fatty acids, was closely related to higher nematode diversity. Increased microbial CO_2 respiration was closely related to a higher nematode channel index. There is a clear inverse relationship between *M. incognita* populations and sweetpotato yield, indicating that high *M. incognita* populations have a very harmful effect on sweetpotato yield.

Discussion

Greenhouse testing

MeloCon was the most efficacious biopesticide, as it supported the lowest populations of *M. incognita* compared to the other bio-based products in greenhouse testing. Similar results were found by Baidoo et al. (2017) on an ornamental shrub where an at-plant treatment of MeloCon resulted in significantly lower *M. incognita* per gram of root than the untreated control. However, they found that MeloCon did not result in higher cut foliage yield or plant growth. We also found that MeloCon did not significantly improve sweetpotato plant biomass. The biopesticide Majestene's performance was similar to MeloCon, supporting statistically similar plant growth and *M. incognita* reproduction. Majestene was selected for field testing due to its activity as a biological nematicide, insecticide, and plant growth promoter. Because of these factors, we hypothesized that Majestene would be a valuable addition to an integrated pest management program.

Velum, the chemical nematicide, performed significantly better than all biopesticides tested in the greenhouse, resulting in the lowest *M. incognita* per gram of root. Chemical nematicides often outperform biopesticides when compared, and this finding is similar to that of Xiang et al. (2017) who found that all plant-growth promoting rhizobacteria strains tested caused lower *M. incognita* mortality when compared with chemical controls aldicarb or abamectin (Hussain et al., 2017).

In the winter cover crop greenhouse test, field peas supported significantly higher *M. incognita* per gram of root than all other winter cover crops tested. This indicates that field peas are a good host of *M. incognita*, with a reproductive factor of 15.3 after 8 weeks of plant growth.

Other research corroborates field pea (*Pisum sativum*) to be an important host of several species of root-knot nematode, including *M. incognita* (Haidar et al., 2009). Crimson clover also supported elevated *M. incognita* populations in the greenhouse, which agrees with findings from Timper et al. (2006) that crimson clover was an excellent host for *M. incognita* under greenhouse conditions. Elbon rye supported the lowest *M. incognita* population per gram of root in the greenhouse, which reinforces previous research that rye is a relatively poor host of *M. incognita* (Timper et al., 2006). The cover crop mixtures examined in these trials supported very low reproductive factors even though they contained the legumes that supported high nematode reproduction. The combination of the grasses and legumes appears to have allowed the benefits of each cover crop (Chapagain et al., 2020).

When testing summer cover crops, McSorley (1999) found that certain legumes like sunn hemp and velvetbean are very desirable because they are highly resistant to *Meloidogyne* spp. and are helpful for nitrogen management. This reinforces our greenhouse finding that the numerically lowest *M. incognita* populations were found on sunn hemp and velvetbean. Wang et al. (2011) also emphasized the utility of sunn hemp as a nematode suppressant cover crop. They found that sunn hemp suppressed plant-parasitic nematode populations for 2 months after cash crop planting, resulting in significantly lower cash crop root gall ratings than the bare ground treatment (Wang et al., 2011). Velvetbean has also been shown to be effective in plant-parasitic nematode management. Weaver, Kabana, and Carden (1998), found that summer cover cropping with velvetbean reduced both *Meloidogyne* spp. and *Heterodera glycines* populations to undetectable levels by fall in both locations tested. Additionally, the low, vining growth habit of velvetbean can aid in weed suppression (Weaver et al., 1998).

Microplot testing

Velum performed best under greenhouse conditions; however, this was not the case in the microplots evaluations where Velum supported the second highest soil *M. incognita* population of all products tested. This is likely due to later sampling timing in the microplots. Colyer et al. (1997) found that the effect of non-fumigant nematicides is typically restricted to the first few weeks after planting, and soil samples were taken in September, three months after Velum was applied. Fluopyram, the active ingredient in Velum, has been reported to have a half-life of 64.2 days in soil, which suggests that the sampling timing did not capture the full potential of its nematicidal activity (Zheng et al., 2014). Further, similar end of the season high nematode populations have been reported with *M. incognita* and *R. reniformis* when aldicarb was applied at crop planting (Lawrence and McLean, 2002; Jones, et al., 2006). In the microplot winter cover crop experiment, field peas supported significantly higher soil *M. incognita* populations at cover crop termination than all other winter cover crops tested. Similarly to its performance in the greenhouse, elbon rye supported significantly lower soil *M. incognita* populations than the fallow, black oats, crimson clover, field peas, yellow mustard, and mix 1 at cover crop termination in the microplots. Previous research has shown that the incorporation of a rye winter cover crop reduces soil populations of *M. incognita* J2's (Johnson and Motsinger, 1989). This reduction has been attributed to rye's production of allelopathic compounds like benzoxazinoids, which degradation products are toxic to *M. incognita* (Zasada et al., 2005; Zasada et al., 2007).

Field testing

Nematodes: All winter cover crops supported more than 10 *M. incognita* J2/100 cm^3 soil which is considered the threshold level at planting (Becker and Westerdahl, 2016). In Alabama, field peas produced the highest biomass but also supported higher *M. incognita* populations than other cover crops. Since field pea is a legume, it adds more nitrogen to the soil, so it may be a

good option for fields without nematode problems (Jackson and Harrison, 2008). Similar higher nematode populations have been observed with *R. reniformis* following legume cover crops of lupins, crimson clover, and vetch (Jones et al., 2006). In North Carolina, the plots following the legume cover crop crimson clover supported numerically elevated *M. incognita* populations in the late season (60 DAP), emphasizing leguminous cover crops' role in maintaining plant-parasitic nematode populations. Jackson and Harrison (2008) affirmed the value of leguminous cover crops in the organic sweetpotato system, mentioning that legume cover crops could potentially fulfill sweetpotato's relatively low nitrogen fertilization needs. University of California at Davis researchers DuPont, Ferris, and Van Horn (2009) found that nematode abundance was 72% higher in cover crop treatments containing legumes than the fallow, and plant productivity was positively associated with legume cover crops in year 2. At the Brewton Agricultural Research Unit, the average yearly precipitation is 168cm (6 inches), however this location experienced yearly precipitation totals of 140cm (55 inches) and 86cm (34 inches) in 2022 and 2023, respectively (Figure 8). Since these were relatively dry years, this impacted soil *M. incognita* populations. At midseason (30 DAP) in both locations, *M. incognita* population density was low across all winter cover crops, probably corresponding to their life cycle with the J2 stage moving out of the soil and colonizing the sweetpotato roots (Moens et al., 2009). At midseason (60 DAP), the plots following crimson clover and field peas had the numerically highest *M. incognita* populations. This suggests a link between leguminous cover crops and increased *M. incognita* population densities, which was also highlighted by Gill et al. (2023) who emphasized that winter legumes like crimson clover and hairy vetch can increase *M. incognita* populations. In general, cereal winter cover crops, like elbon rye, are more effective than leguminous winter cover crops for nematode suppression (Wang et al., 2004). Elbon rye's

deleterious effect on soil *M. incognita* populations was observed at the pre-sweetpotato harvest sampling in North Carolina. The plots following elbon rye numerically supported the lowest soil *M. incognita* populations, a 63% decrease compared to the fallow.

Nematode community: The composition of the soil nematode community can provide valuable insights into the health of the soil ecosystem, since changes in agricultural management practices, like winter cover cropping, can influence the nematode community structure (Bongers and Bongers, 1998). However, no distinct differences in nematode community composition between winter cover crops were observed. Two growing seasons may not be enough time to detect winter cover crop induced changes to the soil nematode community (Blanco-Canqui, et al, 2015). Additionally, sweetpotato production requires intense disturbance to the soil from planting, which requires hilling or bed mounting, to harvest, which requires deep digging (Agbede and Adekiya, 2009). These soil disturbances may have overshadowed changes to the soil nematode community due to winter cover cropping (Wang et al., 2022). Overall, it was clear that upon the conclusion of two years sweetpotato cultivation and winter cover cropping, there were no distinct differences between the winter cover crops tested.

Insect damage: In both Alabama and North Carolina, the primary insect pests belonged to the WDS complex (wireworm, *Diabrotica* spp., and *Systena* spp.). In Alabama, across winter cover crops, damage by this pest complex was similar, although highest following daikon radish. This may be due to the similarity in root structure. The radishes may have acted as a green bridge providing food and habitat for insect pests to survive during a time that the soil is typically left fallow (Jackson and Harrison, 2008). These green bridges allow higher larval insect pest survival through the winter which could increase the risk of damage to the following sweetpotato crop (Favetti et al., 2017). A similar trend was observed with daikon radish cover cropping leading to

increased sweetpotato flea beetle damage to the following sweetpotato crop. The daikon radish cover crop could have provided the green bridge or winter food source for the sweetpotato flea beetle in our trials since the radish produces a large root present over the winter months. The addition of biopesticides statistically reduced WDS complex, sweetpotato flea beetle, and total insect damage in Alabama and WDS complex and total insect damage in North Carolina. In both locations, we saw significantly fewer incidences of WDS damage when BotaniGard 22WP, Triple Threat Entomopathogenic Nematodes, and Majestene were applied. This is similar to Huseth et al. (2021) who found that a tank mix of Majestene and Brigade resulted in fewer WDS holes than the untreated.

Yield: In both locations, we saw a numeric sweetpotato yield benefit when applying the biopesticides (BotaniGard 22 WP, Triple Threat Entomopathogenic Nematodes, and Majestene) when the sweetpotatoes were challenged with *M. incognita* nematodes. Researchers at the University of Georgia also found that Majestene produced significantly higher squash yield in field with *M. incognita* nematode populations (Nnamdi et al., 2022). This finding is like that of Watson et al. (2023) in Louisiana who observed that Majestene resulted in a higher yield of sweetpotato grade number ones under *R. reniformis* nematode pressure. This could be due to Majestene's plant growth promoting effects, which has been documented with other nematicides including aldicarb (Reddy et al., 1990). This trend toward plant growth promotion was also seen in our greenhouse test where the plants treated with Majestene had a 6% greater biomass compared with the untreated control. Entomopathogenic nematodes have also had yield enhancing effects. In cotton, the application of EPNs *S. carpocapsae* and *H. bacteriophora* resulted in increased cotton yield compared to the untreated control (Nagachandrabose, 2012).

Soil health: The Solvita CO_2 respiration test is a simple and quick method to quantify microbial activity in soils and track the results of management changes (Haney et al., 2008). Higher CO_2 respiration is related to the amount or quality of organic carbon and nitrogen in the soil and is considered an indicator of biological attributes linked to healthy soil functioning (Haney et al., 2008). Chahal and Van Eerd (2019) found that Solvita CO_2 burst values were lowest with a no cover crop treatment compared with oilseed radish and rye, which supports our findings that the fallow was consistently among the lowest treatments for soil CO_2 respiration. Blanco-Canqui and others (2015) also emphasized the increase in soil microbial activity with the use of cover crops. In Alabama at sweetpotato planting, the AL mix (crimson clover, field pea, yellow mustard, black oat, daikon radish, and elbon rye) numerically produced the highest microbial CO_2 respiration, indicating that the soil was more biologically active following the mix of legume and grass cover crops. This trend continued at the mid-season and near harvest sampling times. Chahal and Van Eerd (2019) also found that their cover crop mixture of oilseed radish and rye produced higher soil CO_2 respiration than either of the cover crops alone. This indicates a collaborative effect when combining winter cover crops. This could be due to the variety of cover crops stimulating more microbes in the soil and creating a richer environment for microbes to thrive. In North Carolina, the NC mix (crimson clover, daikon radish, elbon rye, and wheat) also performed well, with the highest microbial CO_2 respiration values at sweetpotato planting and at near harvest. This indicates a higher maximal biological activity under these cover crop mixes, which relates to soil health. However, the effects of cover cropping on soil carbon concentration is often not detectable in the first few years after establishment (Blanco-Canqui et al., 2015).

Based on the canonical analysis, sweetpotato yield was positively related to the ratio of gram-positive to gram-negative bacteria. Since the presence of gram-negative bacteria, like *Bacillus* spp., are associated with hardy environments, this indicates that sweetpotatoes yield better in a more resilient soil environment (Paudel et al., 2021). This data also suggests that sweetpotato yield is enhanced when soil food web structure is less disturbed, as indicated by a high nematode structural index (Du Preez et al., 2022). The relationship between microbial CO_2 respiration and high saprophytic fungal biomass suggests that the majority of microbial respiration was dominated by fungal decomposition (Paudel et al., 2021). More abundant total microbial biomass (measured as total phospholipid fatty acids) was closely related to higher nematode diversity, which shows that higher microbial biomass can support the flourishing of a variety of free-living nematodes, and an increase in soil health (Paudel et al., 2021). Additionally, the analysis shows an acute inverse relationship between sweetpotato yield and *M. incognita* populations. This clearly indicates the major importance of managing plant-parasitic nematode populations to achieve high sweetpotato yields, as emphasized by Ploeg et al. (2019).

Summary: The objectives of this work were (1) to evaluate biopesticides for the management of *M. incognita* and insect pests, (2) to determine the efficacy of winter cover crops in the suppression of *M. incognita* populations and insect pest damage, and (3) to assess the impact of winter cover crops on soil health indicators. Our findings indicate that the combination of BotaniGard 22WP, Triple Threat Entomopathogenic Nematodes, and Majestene significantly reduced insect damage to sweetpotatoes under field conditions. The winter cover crops elbon rye and the mix containing crimson clover, daikon radish, elbon rye, and wheat resulted in lowered soil *M. incognita* populations when compared with leguminous winter cover crops like field peas and crimson clover. Total insect pest damage was similar across winter cover crops, but lowest

following crimson clover in North Carolina and black oats in Alabama. Thus, location can make a difference in cover crop effects. Soil health values measured by the Solvita CO_2 Burst test were elevated following the winter cover crop mixes compared with the single winter cover crop treatments, indicating that the mixes stimulate higher maximal biological activity, which relates to soil health.

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Table 1. Effect of biopesticides on sweetpotato plant growth parameters and *Meloidogyne incognita* race 3 nematode reproduction when grown in the greenhouse.

Biological control			Root fresh weight (g)		Shoot fresh weight (g)		Biomass[a] (g)		*M incognita* eggs/ root system		*M. incognita* eggs/ g root[b]	
Name	Active ingredient	Rate										
Untreated	None	None	36	a[c]	35	a	71	a	114,778	ab	3,607	ab
AzaGuard	Azadirachtin	1.1 L/ha	37	a	32	a	69	a	83,183	ab	2,316	ab
BotaniGard 22 WP + Triple Threat Entomopathogenic Nematodes	*Beauveria bassiana* + *Steinernema feltiae, Steinernema carpocapsae, Heterorhabditis bacteriophora*	2.3 L/ha	39	a	31	a	70	a	151,634	a	5,180	a
BoteGHA ES	*Beauveria bassiana*	4.9 kg/ha + 123.5 million IJ's/ha	42	a	37	a	80	a	121,832	ab	3,039	ab
Chitocide	Quillaja extract and chitosan	1.2 kg/ha	43	a	31	a	74	a	97,874	ab	2,817	ab
Majestene	Heat-killed *Burkholderia spp.*	18.7 L/ha	37	a	35	a	76	a	129,140	ab	4,657	a
MeloCon	*Purpureocillium lilacinum*	0.7 L/ha	40	a	35	a	75	a	73,018	ab	2,086	ab
Minuet	*Bacillus subtilis*	1.5 L/ha	39	a	33	a	72	a	96,509	ab	2,813	ab
Monterey Nematode Control	Saponins of *Quillaja saponaria*	2.5 L/ha	37	a	35	a	73	a	132,973	ab	4,189	ab
Promax	Thyme oil	12.4 L/ha	42	a	35	a	77	a	134,160	a	3,929	ab
Seduce	Spinosad	33.6 kg/ha	49	a	34	a	84	a	117,961	ab	2,806	ab
Velum	Fluopyram	0.4 L/ha	43	a	33	a	72	a	7,563	b	194	b
P value[d]			0.2123		0.9370		0.7363		0.0239**		0.0375**	

[a] Biomass is the sum of root and shoot fresh weight in grams.

[b] *Meloidogyne incognita* eggs/g of fresh root weight.

[c] Values followed by the same letter are not significantly different at $P \leq 0.05$ as determined by the Tukey Kramer Method.

[d] *P*-values for Type III fixed effects with significance at the 0.1, 0.05, 0.01, and 0.001 level is indicated by *, **, ***, and **** respectively.

Table 2. Effect of winter and summer cover crops on plant growth and *Meloidogyne incognita* race 3 reproduction in the greenhouse.

Winter cover crop	Root fresh weight (g)		Shoot fresh weight (g)		Biomass[a] (g)		*Meloidogyne incognita* eggs/ g root[b]		Reproductive factor[c]
Fallow	0	c[d]	0	d	0	d	0	b	0.2
Black oats	141	ab	26	b	167	ab	105	b	1.6
Crimson clover	16	c	13	c	29	d	1,834	b	5.0
Daikon radish	76	bc	45	a	121	bc	79	b	1.0
Elbon rye	166	a	16	d	182	ab	48	b	1.1
Field peas	24	c	17	c	41	cd	5,430	a	15.3
Wheat	145	ab	18	c	162	ab	160	b	2.5
Yellow mustard	36	c	29	b	65	cd	352	b	1.5
Mix 1[e]	171	a	29	b	200	a	51	b	1.1
Mix 2[f]	170	a	21	bc	191	ab	62	b	1.0
P value[g]	0.0001****		0.0001****		0.0001****		0.0001****		
Summer cover crop									
Fallow	0	c	0	d	0	c	0	b	0.002
Elbon rye	182	ab	30	c	213	ab	288	a	0.058
Piper sudangrass	244	a	58	ab	303	a	176	ab	0.035
Sunn hemp	130	b	50	b	181	b	23	ab	0.005
Velvetbean	101	b	70	a	171	b	18	b	0.004
P value[g]	0.0001****		0.0001****		0.0001****		0.0136**		

[a] Biomass is the sum of root and shoot fresh weight in grams.
[b] *Meloidogyne incognita* eggs/g of fresh root weight.
[c] Calculated by dividing the final population of *M. incognita* by its initial population.
[d] Values followed by the same letter are not significantly different at $P \leq 0.05$ as determined by the Tukey Kramer Method.
[e] Mix 1 contained crimson clover, field pea, yellow mustard, black oat, daikon radish, and elbon rye.
[f] Mix 2 contained crimson clover, daikon radish, elbon rye, and wheat.
[g] *P*-values for Type III fixed effects with significance at the 0.1, 0.05, 0.01, and 0.001 level is indicated by *, **, ***, and **** respectively.

Table 3. Effect of biopesticides on *Meloidogyne incognita* race 3 reproduction and sweetpotato yield in a microplot setting.

Biopesticide			*M. incognita*/ 100 cm^3 soil		Marketable yield/ plant (g)	
Name	Active ingredient	Rate				
Untreated	None	None	1,236	a[a]	508.7	a
AzaGuard	Azadirachtin	1.1 L/ha	1,082	c	512.5	a
BoteGHA ES	*Beauveria bassiana*	2.3 L/ha	1,190	ab	441.7	a
BotaniGard 22WP + Triple Threat Entomopathogenic Nematodes	*Beauveria bassiana* + *Steinernema feltiae, Steinernema carpocapsae, Heterorhabditis bacteriophora*	4.9 kg/ha + 123.5 million IJ's/ha	773	f	533.2	a
Chitocide	Quillaja extract and chitosan	1.2 kg/ha	819	e	558.4	a
Majestene	Heat-killed *Burkholderia spp.*	18.7 L/ha	1,043	c	505.1	a
MeloCon	*Purpureocillium lilacinum*	0.7 L/ha	765	f	377.5	a
Minuet	*Bacillus subtilis*	1.5 L/ha	881	d	459.5	a
Monterey Nematode Control	Saponins of *Quillaja saponaria*	2.5 L/ha	920	d	367.4	a
Promax	Thyme oil	12.4 L/ha	834	e	431.7	a
Seduce	Spinosad	33.6 kg/ha	819	e	418.4	a
Velum	Fluopyram	0.4 L/ha	1,159	b	517.4	a
P value[b]			0.0001****		0.9743	

[a] Values followed by the same letter are not significantly different at $P \leq 0.05$ as determined by the Tukey Kramer Method.

[b] *P*-values for Type III fixed effects with significance at the 0.1, 0.05, 0.01, and 0.001 level is indicated by *, **, ***, and **** respectively.

Table 4. Effect of winter cover crops on *Meloidogyne incognita* race 3 reproduction and sweetpotato yield in a microplot setting.

Winter cover crop	Winter cover crop biomass[a] (g)		April *M. incognita* J2's / 100cm^3 soil		July *M. incognita* J2's / 100cm^3 soil		September *M. incognita* J2's/ 100cm^3 soil		Sweetpotato yield (kg/ plant)	
Fallow	18	d[b]	30	ab	421	a	470	a	0.90	a
Black oats	51	bcd	22	dc	305	a	333	a	1.43	a
Crimson clover	44	bcd	19	dc	570	a	407	a	0.92	a
Daikon radish	32	dc	17	d	282	a	437	a	1.36	a
Elbon rye	70	abc	18	d	330	a	317	a	1.30	a
Field peas	97	a	35	a	416	a	385	a	1.05	a
Yellow mustard	27	d	26	bc	879	a	507	a	1.10	a
Mix 1[c]	84	ab	21	dc	393	a	317	a	1.38	a
P value[d]	0.0001****		0.0001****		0.0661*		0.0001****		0.2458	

[a] Winter cover crop biomass was assessed as dry weight of aboveground biomass.
[b] Values followed by the same letter are not significantly different at $P \leq 0.1$ as determined by the Tukey Kramer Method.
[c] Mix 1 contained crimson clover, daikon radish, elbon rye, and wheat.
[d] *P*-values for Type III fixed effects with significance at the 0.1, 0.05, 0.01, and 0.001 level is indicated by *, **, ***, and **** respectively.

Table 5. *Meloidogyne incognita* populations throughout the sweetpotato cropping following the winter cover crops in Alabama, 2022-2023.

Winter cover crop	Cover crop biomass[a] (kg/ha)		At plant *M. incognita* J2's / 100cm^3 soil		30 DAP *M. incognita* J2's / 100cm^3 soil		60 DAP *M. incognita* J2's / 100cm^3 soil		84 DAP *M. incognita* J2's / 100cm^3 soil	
Winter cover crop										
Fallow	0	c[b]	29	a	1	b	43	a	207	a
Black oats	3,269	bc	29	a	3	b	42	a	361	a
Crimson clover	3,889	b	48	a	16	a	87	a	281	a
Daikon radish	5,352	ab	10	a	6	ab	35	a	273	a
Elbon rye	3,363	bc	19	a	1	b	29	a	275	a
Field peas	8,685	a	193	a	1	b	77	a	424	a
Yellow mustard	3,294	bc	48	a	5	ab	45	a	220	a
AL mix	7,484	ab	61	a	1	b	32	a	375	a
P value[c]	0.0001****		0.1635		0.0096***		0.1886		0.4883	

[a] Winter cover crop biomass was assessed as dry weight of aboveground biomass.
[b] Values followed by the same letter are not significantly different at $P \leq 0.1$ as determined by the Tukey Kramer Method.
[c] *P*-values for Type III fixed effects with significance at the 0.1, 0.05, 0.01, and 0.001 level is indicated by *, **, ***, and **** respectively.

Table 6. Sweetpotato root damage due to WDS complex, white grubs, flea beetles, and *M. incognita* in Alabama, 2022-2023.										
Source of variation (F-value)	WDS complex damage[a]		White grub damage[b]		Sweetpotato Flea beetle damage[c]		*M. incognita* damage[d]		Total insect damage[e]	
Winter cover crop	0.7612		0.1051		0.1518		0.1620		0.5236	
Biopesticides	0.0528*		0.2323		0.0294**		0.3411		0.0172**	
Winter cover crop x Biopesticides	0.9944		0.6847		0.5274		0.9150		0.9780	
Biopesticides[f]										
Untreated	9.26	A[g]	0.22	a	2.23	a	0.11	a	11.71	a
Treated	7.83	b	0.16	a	1.87	b	0.07	a	9.86	b
P value[h]	0.0466**		0.2119		0.0627*		0.2418		0.0180**	
Winter cover crop										
Fallow	8.34	a	0.28	ab	2.16	a	0.03	a	10.78	a
Black oats	7.21	a	0.14	ab	1.29	a	0.08	a	8.64	a
Crimson clover	9.03	a	0.31	a	1.93	a	0.10	a	11.26	a
Daikon radish	9.74	a	0.15	ab	2.34	a	0.04	a	12.23	a
Elbon rye	8.44	a	0.21	ab	2.29	a	0.08	a	11.94	a
Field peas	7.98	a	0.25	ab	2.23	a	0.19	a	10.46	a
Yellow mustard	9.16	a	0.06	b	2.06	a	0.05	a	11.29	a
AL mix	8.48	a	0.14	ab	2.12	a	0.16	a	10.70	a
P value[h]	0.7326		0.0965*		0.1471		0.1407		0.4872	

[a] WDS complex consists of wireworm spp., *Diabrotica* spp., and *Systena* spp. and damage was assessed as average number of insect holes per sweetpotato.

[b] White grub (*Phyllophaga* spp.) damage consists of wide tunnels gouged into the surface of sweetpotato roots.

[c] Sweetpotato flea beetle (*Chaetocnema* spp.) damage consists of thin winding tunnels etched into the sweetpotato periderm.

[d] *Meloidogyne incognita* damage consists of root cracking.

[e] Total insect damage is expressed as the sum of WDS complex, white grub, and flea beetle damage which was assessed as average number of insect damage incidences per sweetpotato.

[f] Biopesticides consisted of Triple Threat Beneficial Nematodes; *Steinernema feltiae, S.carpocapsae,* and *Heterorhabditis bacteriophora* at 123.5 million IJ's/ha,BotaniGard 22 WP; *Beauveria bassiana* strain GHA at 4.9 kg/ha; and Majestene; heat-killed Burkholderia spp. strain A396 cells and spent fermentation media 18.7 L/ha.

[g] Values followed by the same letter are not significantly different at $P \leq 0.1$ as determined by the Tukey Kramer Method.

[h] *P*-values for Type III fixed effects with significance at the 0.1, 0.05, 0.01, and 0.001 level is indicated by *, **, ***, and **** respectively.

Table 7. Sweetpotato numbers, yields (kg/ha) and economic value by quality grade with and without biopesticides following winter cover crops in Alabama, 2022-2023.

Source of variation (F-value)	Jumbo count[a] (number/ ha)		Jumbo weight (kg/ ha)		Number ones count (number/ ha)		Number ones weight (kg/ ha)		Canners count (number/ ha)		Canners weight (kg/ ha)		Total marketable yield[b] (kg/ ha)		Value of marketable yield ($/ ha)	
Winter cover crop	0.4574		0.1312		0.2612		0.2612		0.8157		0.7970		0.1552		0.6302	
Biopesticides	0.5505		0.8272		0.3997		0.3997		0.0131**		0.0131**		0.2010		0.4995	
Winter cover crop x Biopesticides	0.6436		0.4426		0.9795		0.9795		0.8450		0.6082		0.9520		0.9982	
Biopesticides[c]																
Untreated	3,662	a[d]	2,919	a	15,702	a	7,122	a	26,956	b	3,978	b	13,863	a	$955	a
Treated	3,354	a	2,832	a	16,477	a	7,474	a	32,129	a	4,732	a	14,882	a	$1,015	a
P value	0.6311		0.8426		0.5030		0.5030		0.0041***		0.0086***		0.2208		0.5627	
Winter cover crop																
Fallow	2,915	a	2,026	a	15,514	a	7,037	a	29,713	a	4,238	a	13,300	a	$889	a
Black oats	2,355	a	1,848	a	16,820	a	7,629	a	30,274	a	4,277	a	13,747	a	$942	a
Crimson clover	3,252	a	2,439	a	15,446	a	7,006	a	31,171	a	4,320	a	13,766	a	$905	a
Daikon radish	3,924	a	3,091	a	16,579	a	7,520	a	27,695	a	4,244	a	14,855	a	$982	a
Elbon rye	4,037	a	3,447	a	17,893	a	8,116	a	31,059	a	4,484	a	16,047	a	$1,063	a
Field peas	4,485	a	3,682	a	19,398	a	8,799	a	31,395	a	4,663	a	17,143	a	$1,146	a
Yellow mustard	3,364	a	2,413	a	14,130	a	6,409	a	30,162	a	4,875	a	13,697	a	$855	a
AL mix	3,735	a	4,069	a	12,937	a	5,868	a	24,872	a	3,739	a	12,428	a	$1,099	a
P value[e]	0.4446		0.1592		0.2281		0.2281		0.8016		0.7906		0.1324		0.5918	

[a] Yield was separated by quality size classification into jumbo, number one, and canner grades.

[b] Total marketable yield is expressed as the sum of the weights of jumbos, number ones, and canners.

[c] Biopesticides consisted of Triple Threat Beneficial Nematodes; *Steinernema feltiae, S.carpocapsae,* and *Heterorhabditis bacteriophora* at 123.5 million IJ's/ha,BotaniGard 22 WP; *Beauveria bassiana* strain GHA at 4.9 kg/ha; and Majestene; heat-killed Burkholderia spp. strain A396 cells and spent fermentation media 18.7 L/ha.

[d] Values followed by the same letter are not significantly different at $P \leq 0.1$ as determined by the Tukey Kramer Method.

[e] *P*-values for Type III fixed effects with significance at the 0.1, 0.05, 0.01, and 0.001 level is indicated by *, **, ***, and **** respectively.

Table 8. Soil CO_2 respiration during the sweetpotato cropping season following the winter cover crops in Alabama, 2022-2023.						
Source of variation (F-value)	At plant soil CO_2 respiration[a] (ppm)		30 DAP soil CO_2 respiration (ppm)		84 DAP soil CO_2 respiration (ppm)	
Winter cover crop						
Fallow	22.3	a[b]	19.7	a	26.5	a
Black oats	23.8	a	20.1	a	30.2	a
Crimson clover	23.5	a	21.3	a	30.7	a
Daikon radish	21.6	a	20.9	a	29.7	a
Elbon rye	21.2	a	23.1	a	33.9	a
Field peas	22.2	a	19.2	a	30.0	a
Yellow mustard	16.7	a	17.7	a	25.1	a
AL mix	38.1	a	26.7	a	32.2	a
P value[c]	0.3085		0.2256		0.1102	

[a] Soil CO_2 respiration was measured using the Solvita CO_2 Burst procedure.
[b] Values followed by the same letter are not significantly different at $P \leq 0.1$ as determined by the Tukey Kramer Method.
[c] *P*-values for Type III fixed effects with significance at the 0.1, 0.05, 0.01, and 0.001 level is indicated by *, **, ***, and **** respectively.

Table 9. *Meloidogyne incognita* populations throughout the sweetpotato cropping season following the winter crop crops in North Carolina 2022-2023.

Winter cover crop	Winter cover crop biomass[a] (kg/ha)		March *M. incognita* / 100cm^3 soil		At plant *M. incognita* / 100cm^3 soil		30 DAP *M. incognita* / 100cm^3 soil		60 DAP *M. incognita* / 100cm^3 soil		84 DAP *M. incognita* / 100cm^3 soil	
Fallow	7,045	d[b]	30	a	15	a	36	a	150	a	134	a
Crimson clover	14,753	cd	46	a	21	a	44	a	116	a	131	a
Daikon radish	10,197	cd	38	a	18	a	44	a	60	a	82	a
Elbon rye	26,404	a	77	a	26	a	77	a	24	a	49	a
Wheat	17,895	bc	31	a	15	a	64	a	81	a	103	a
NC mix	24,403	ab	23	a	21	a	64	a	54	a	225	a
P value[c]	0.0001****		0.4982		0.9480		0.1792		0.6640		0.6580	

[a] Winter cover crop biomass was assessed as dry weight of aboveground biomass.
[b] Values followed by the same letter are not significantly different at $P \leq 0.1$ as determined by the Tukey Kramer Method.
[c] *P*-values for Type III fixed effects with significance at the 0.1, 0.05, 0.01, and 0.001 level is indicated by *, **, ***, and **** respectively.

Table 10. Sweetpotato root damage due to WDS complex, white grubs, sweetpotato flea beetles, and *M. incognita* in North Carolina, 2022-2023.

	WDS complex damage[a]		White grub damage[b]		Sweetpotato flea beetle damage[c]		*M. incognita* damage[d]		Total insect damage[e]	
Winter cover crop	0.3236		0.9132		0.4519		0.6056		0.3512	
Biopesticide	0.0001***		0.4706		0.6931		0.2910		0.0001***	
Winter cover crop x Biopesticide	0.6979		0.1851		0.4018		0.9508		0.5070	
Biopesticide[f]										
Untreated	3.54	a[g]	0.21	a	0.08	a	0.01	a	3.83	a
Treated	2.13	b	0.25	a	0.07	a	0.03	a	2.45	b
P value[h]	0.0001****		0.4759		0.6933		0.2825		0.0001****	
Winter cover crop										
Fallow	3.53	a	0.17	a	0.13	a	0.03	a	3.83	a
Crimson clover	2.43	a	0.23	a	0.06	a	0	a	2.72	a
Daikon radish	2.53	a	0.27	a	0.08	a	0.03	a	2.88	a
Elbon rye	2.58	a	0.25	a	0.07	a	0.03	a	2.89	a
Wheat	3.21	a	0.27	a	0.04	a	0	a	3.52	a
NC mix	2.72	a	0.19	a	0.07	a	0.05	a	2.98	a
P value[h]	0.3127		0.9173		0.4526		0.5865		0.3472	

[a] WDS complex consists of wireworm spp., *Diabrotica* spp., and *Systena* spp. and damage was assessed as average number of insect holes per sweetpotato.

[b] White grub (*Phyllophaga* spp.) damage consists of wide tunnels gouged into the surface of sweetpotato roots.

[c] Sweetpotato flea beetle (*Chaetocnema* spp.) damage consists of thin winding tunnels etched into the sweetpotato periderm.

[d] *M. incognita* damage consists of root cracking.

[e] Total insect damage is expressed as the sum of WDS complex, white grub, and flea beetle damage which was assessed as average number of insect damage incidences per sweetpotato.

[f] Biopesticides consisted of Triple Threat Beneficial Nematodes; *Steinernema feltiae, S.carpocapsae,* and *Heterorhabditis bacteriophora* at 123.5 million IJ's/ha,BotaniGard 22 WP; *Beauveria bassiana* strain GHA at 4.9 kg/ha; and Majestene; heat-killed Burkholderia spp. strain A396 cells and spent fermentation media 18.7 L/ha.

[g] Values followed by the same letter are not significantly different at $P \leq 0.1$ as determined by the Tukey Kramer Method.

[h] *P*-values for Type III fixed effects with significance at the 0.1, 0.05, 0.01, and 0.001 level is indicated by *, **, ***, and **** respectively.

Table 11. Sweetpotato numbers, yields (kg/ha) and economic value by quality grade with and without biopesticides following winter cover crops in North Carolina, 2022-2023.

Source of variation (F-value)	Jumbo count[a] (number/ ha)		Jumbo weight (kg/ ha)		Number ones count (number/ ha)		Number ones weight (kg/ ha)		Canners count (number/ ha)		Canners weight (kg/ ha)		Total marketable yield[b] (kg/ ha)		Value of marketable yield ($/ ha)	
Winter cover crop	0.7491		0.5999		0.5687		0.5971		0.0552*		0.0952*		0.8652		0.9315	
Biopesticide[c]	0.8058		0.4553		0.9277		0.7311		0.8832		0.5714		0.4921		0.8255	
Winter cover crop x Biopesticide	0.2757		0.1654		0.9189		0.9375		0.4834		0.6174		0.9149		0.9798	
Biopesticide																
Untreated	3,707	a[d]	3,245	a	37,358	a	11,720	a	33,627	a	3,947	a	18,913	a	$1,781	a
Treated	3,827	a	3,575	a	37,167	a	11,972	a	33,914	a	4,113	a	19,659	a	$1,814	a
P value[e]	0.8070		0.4613		0.9621		0.8193		0.8867		0.5864		0.5597		0.8161	
Winter cover crop																
Fallow	3,803	a	3,366	a	37,669	a	11,764	a	28,557	b	3,289	a	18,419	a	$1,793	a
Crimson clover	4,520	a	4,180	a	34,225	a	10,978	a	33,149	ab	3,717	a	18,875	a	$1,709	a
Daikon radish	3,516	a	3,343	a	37,238	a	12,517	a	31,714	ab	3,851	a	19,711	a	$1,864	a
Elbon rye	3,229	a	2,760	a	38,601	a	11,926	a	37,597	ab	4,555	a	19,241	a	$1,800	a
Wheat	3,875	a	3,547	a	40,539	a	12,870	a	33,651	ab	4,263	a	20,679	a	$1,930	a
NC mix	3,659	a	3,265	a	35,301	a	11,021	a	37,956	a	4,503	a	18,789	a	$1,688	a
P value[e]	0.7544		0.6144		0.9562		0.8965		0.0693*		0.1219		0.9289		0.9257	

[a] Yield was separated by quality size classification into jumbo, number one, and canner grades.

[b] Total marketable yield is expressed as the sum of the weights of jumbos, number ones, and canners.

[c] Biopesticides consisted of Triple Threat Beneficial Nematodes; *Steinernema feltiae, S.carpocapsae,* and *Heterorhabditis bacteriophora* at 123.5 million IJ's/ha,BotaniGard 22 WP; *Beauveria bassiana* strain GHA at 4.9 kg/ha; and Majestene; heat-killed Burkholderia spp. strain A396 cells and spent fermentation media 18.7 L/ha.

[d] Values followed by the same letter are not significantly different at $P \leq 0.1$ as determined by the Tukey Kramer Method.

[e] *P*-values for Type III fixed effects with significance at the 0.1, 0.05, 0.01, and 0.001 level is indicated by *, **, ***, and **** respectively.

Table 12. Soil CO_2 respiration during the sweetpotato cropping season following the winter cover crops in North Carolina, 2022-2023.

Winter cover crop	At plant soil CO_2 respiration[a] (ppm)		30 DAP soil CO_2 respiration (ppm)		84 DAP soil CO_2 respiration (ppm)	
Winter cover crop						
Fallow	57.4	a[b]	79.2	ab	84.5	a
Crimson clover	54.6	a	96.5	a	88.1	a
Daikon radish	56.0	a	78.6	ab	76.3	a
Elbon rye	61.5	a	69.6	b	77.6	a
Wheat	65.5	a	84.1	ab	95.2	a
NC mix	69.2	a	95.3	a	95.2	a
P value[c]	0.8920		0.0258**		0.2310	

[a] Soil CO_2 respiration was measured using the Solvita CO_2 Burst procedure.
[b] Values followed by the same letter are not significantly different at $P \leq 0.1$ as determined by the Tukey Kramer Method.
[c] *P*-values for Type III fixed effects with significance at the 0.1, 0.05, 0.01, and 0.001 level is indicated by *, **, ***, and **** respectively.

Figure 1. Sweetpotato grade classifications of jumbo, number one, canner, and cull from left to right.

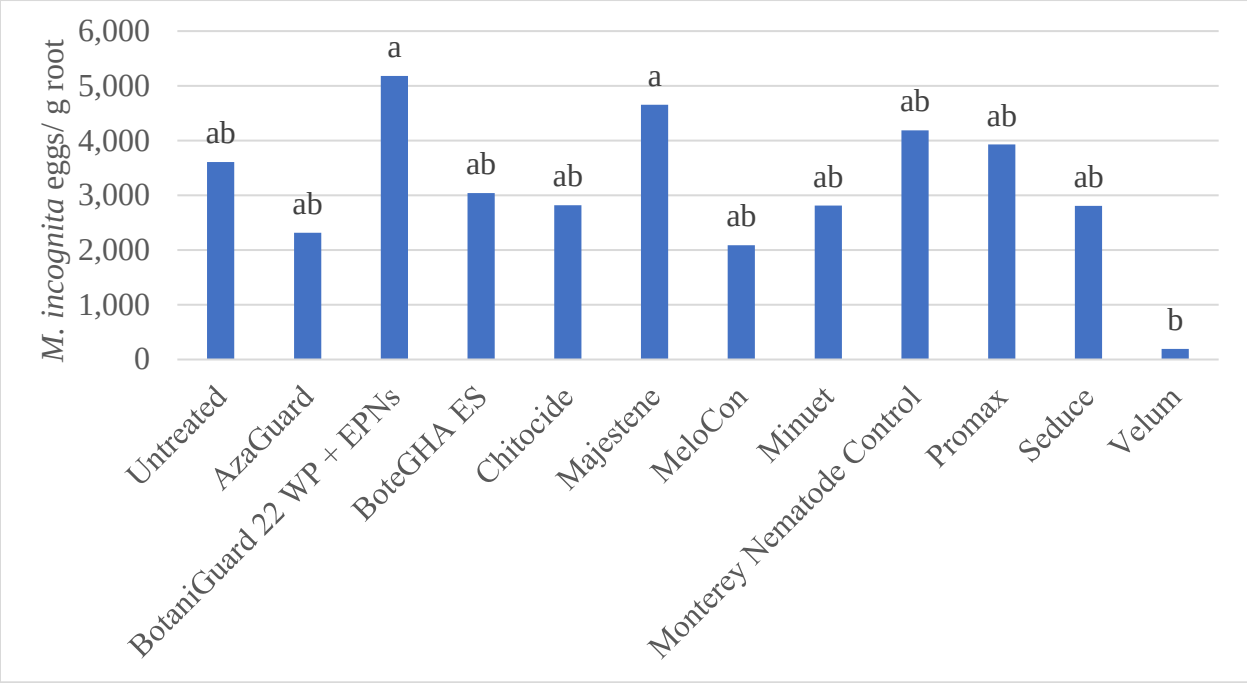


Figure 2. Effect of biopesticides on *Meloidogyne incognita* race 3 reproduction under greenhouse conditions.

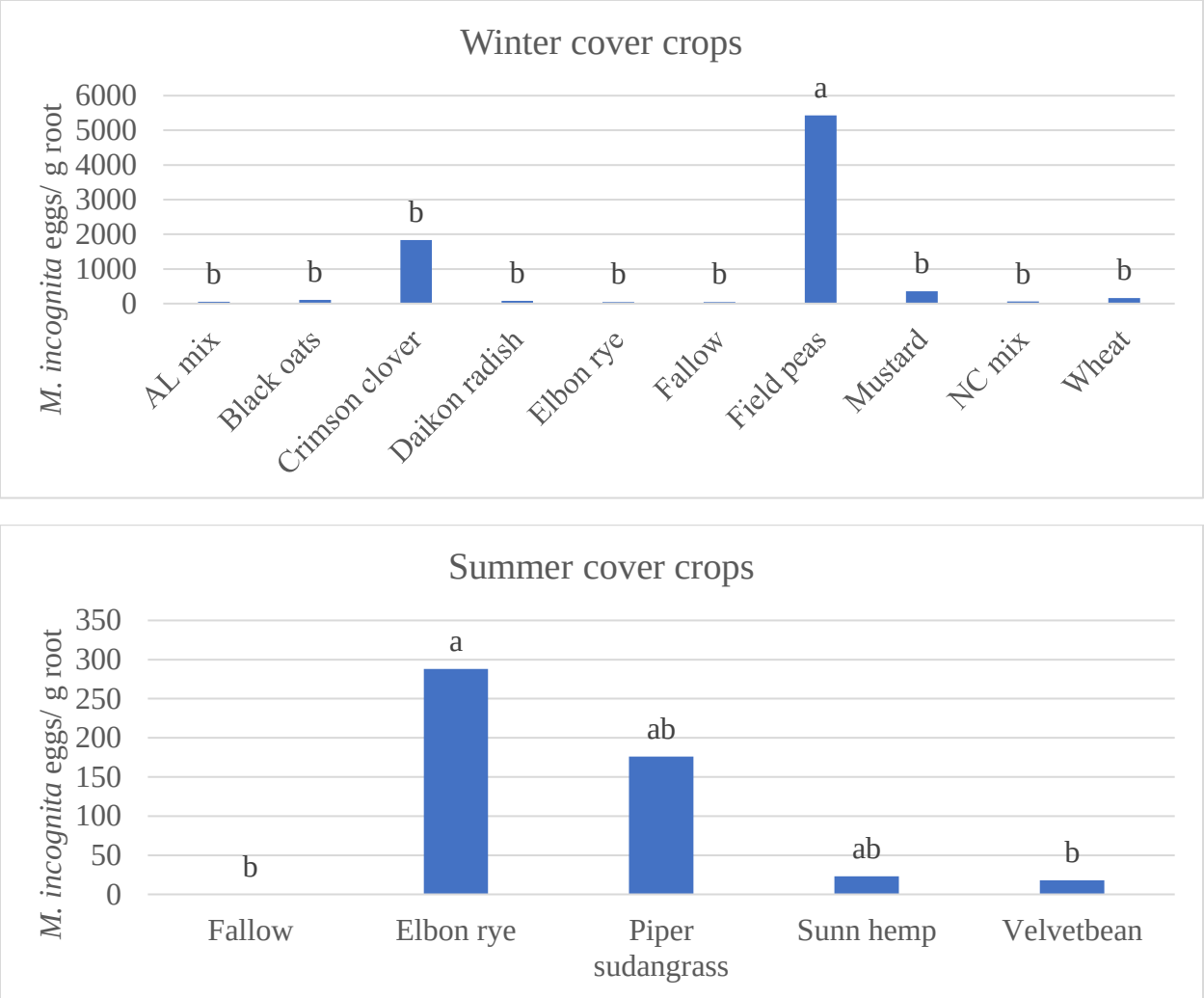


Figure 3. Effect of selected winter (above) and summer (below) cover crops on *Meloidogyne incognita* race 3 reproduction under greenhouse conditions.

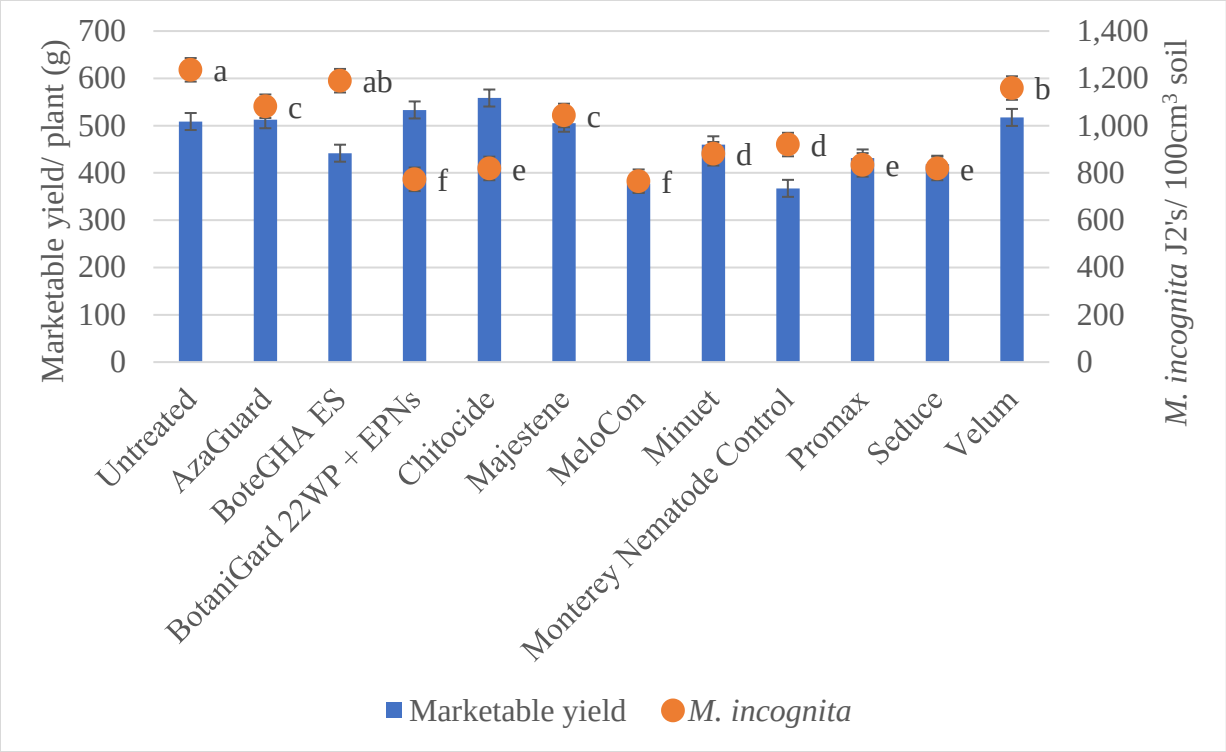


Figure 4. Effect of biopesticides and *Meloidogyne incognita* race 3 on sweetpotato marketable yield in a microplot setting.

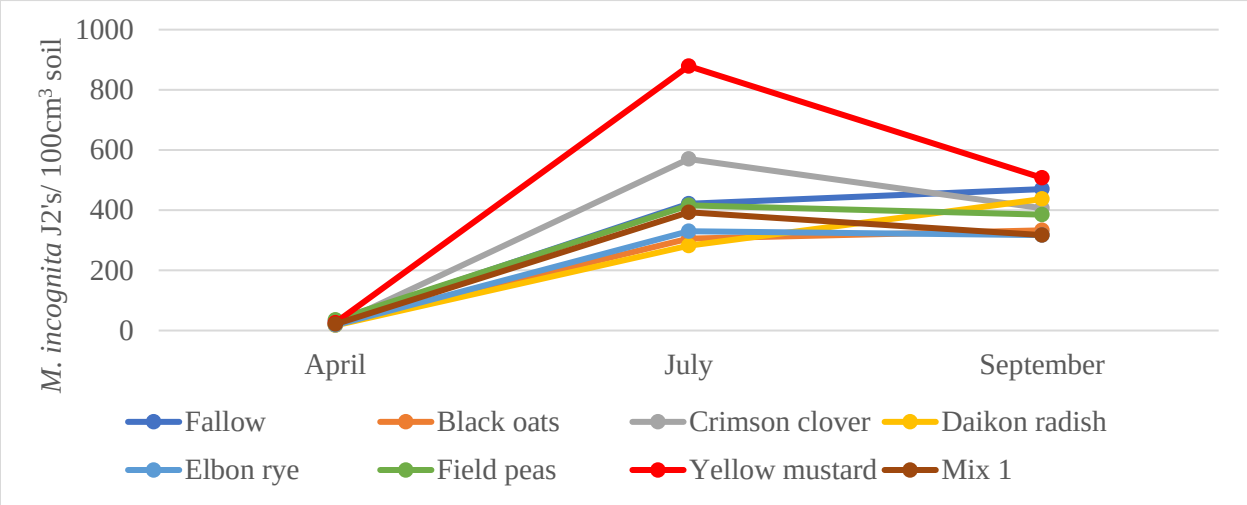


Figure 5. Effect of winter cover crops on *Meloidogyne incognita* race 3 reproduction in a microplot setting, 2023.

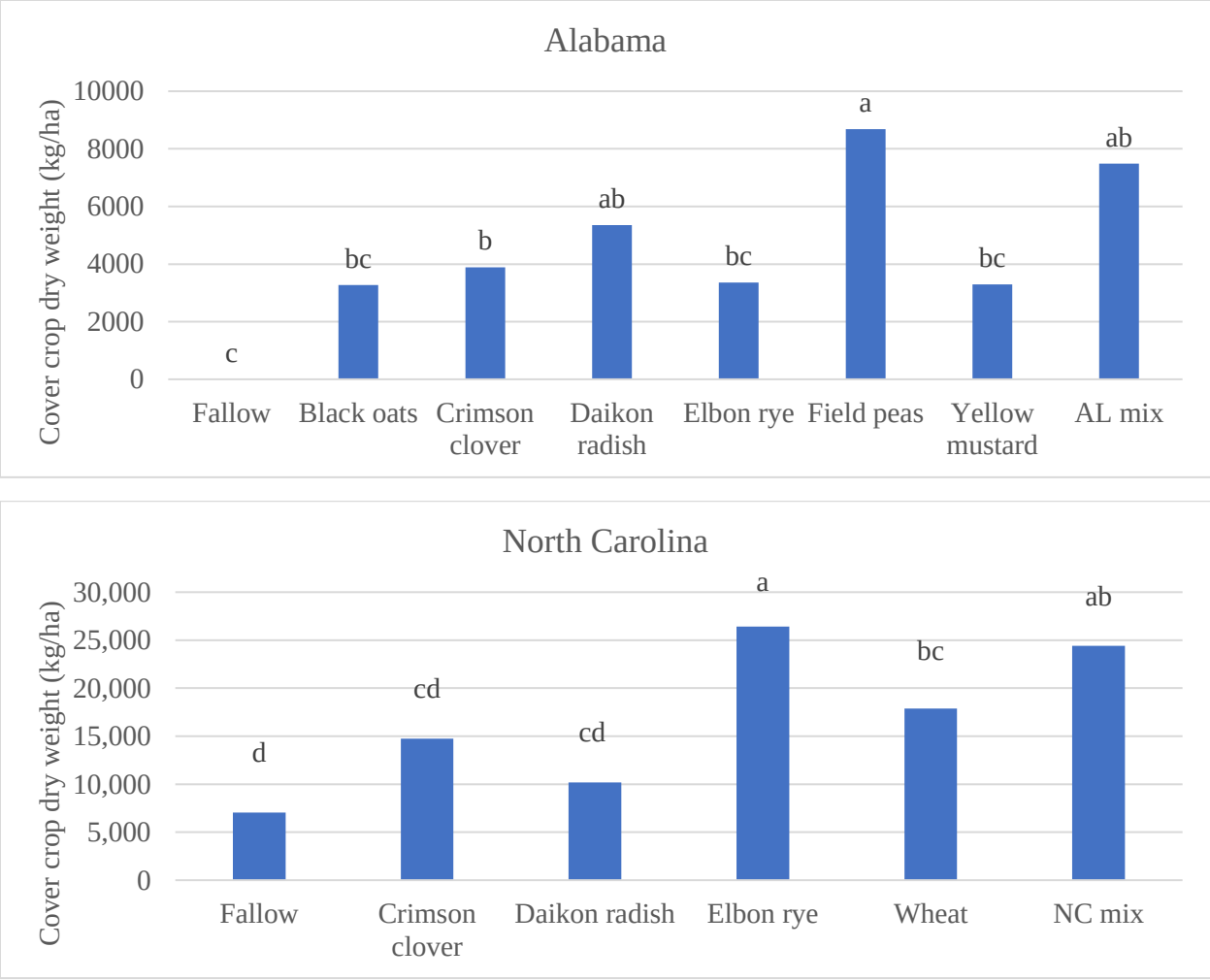


Figure 6. Winter cover crop biomass measured as shoot dry weight at cover crop termination, Alabama (above) and North Carolina (below), 2022-2023.

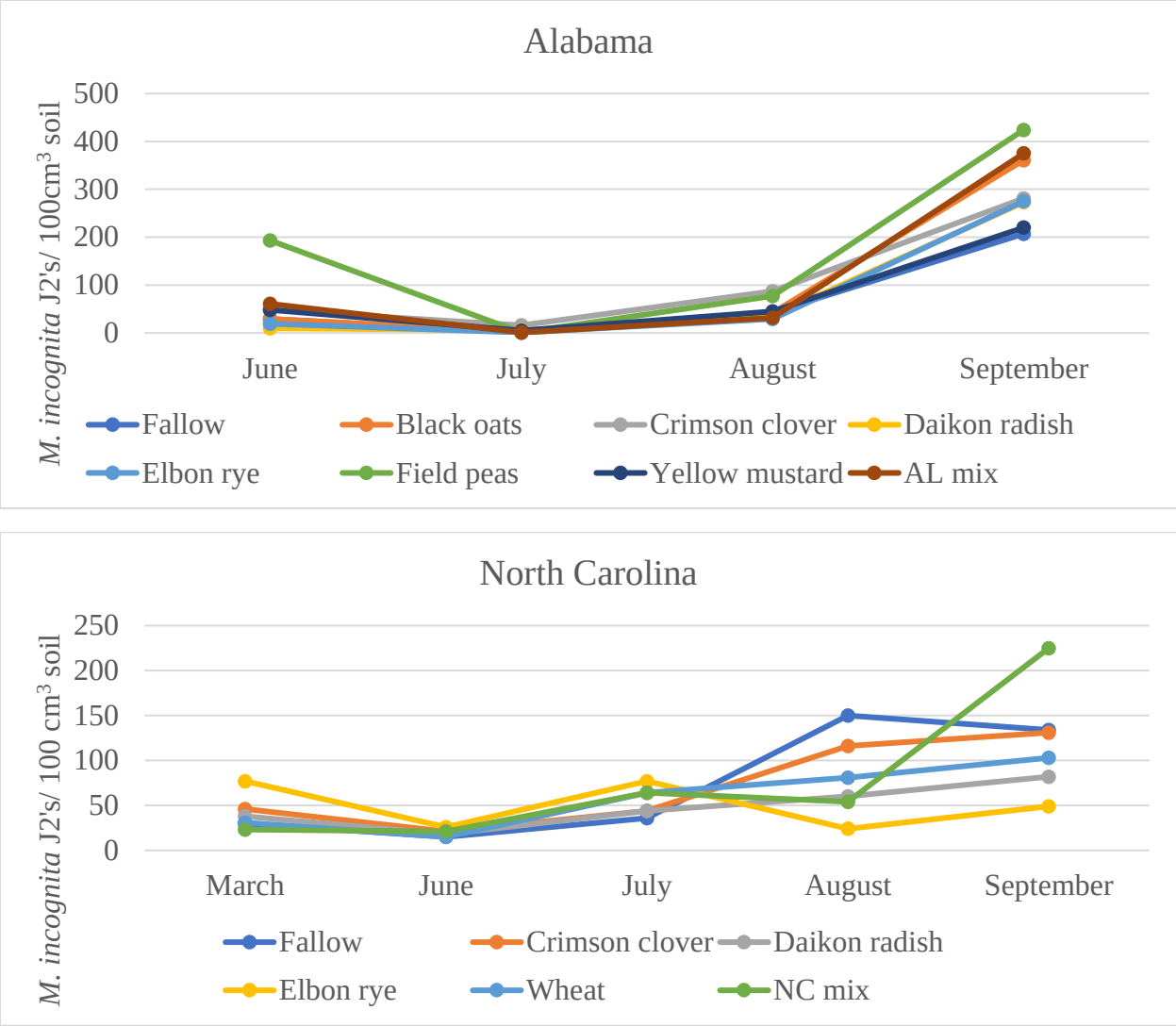
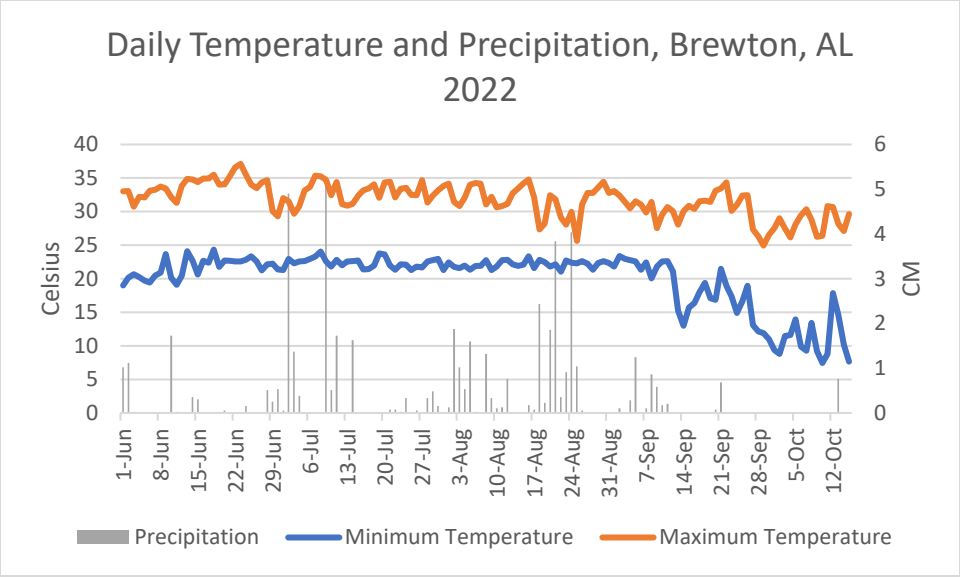


Figure 7. Soil *Meloidogyne incognita* population densities across the sweetpotato cropping season following the winter cover crops in Alabama (above) and North Carolina (below) 2022-2023.



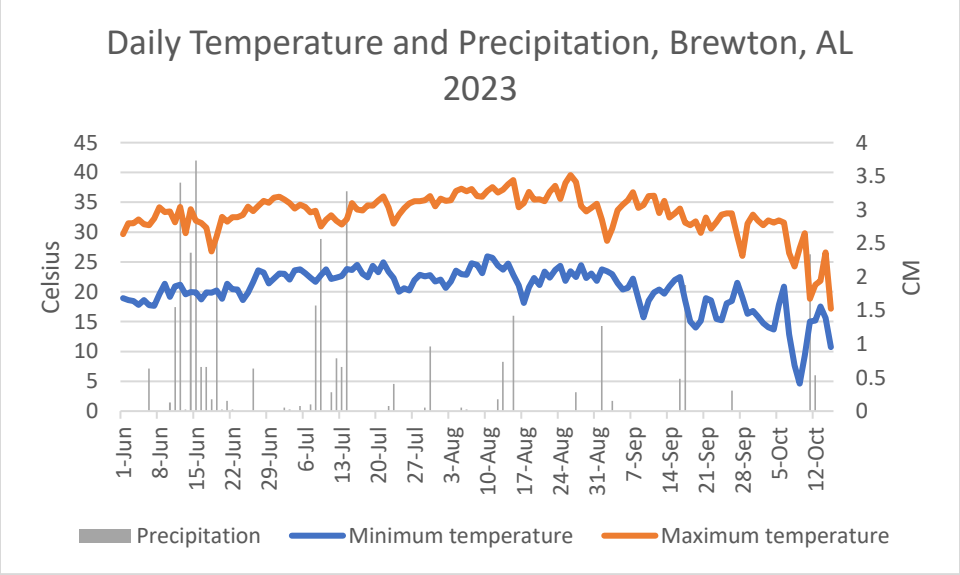


Figure 8. Daily temperature and precipitation at Brewton Agricultural Research Unit, 2022 and 2023. Data were retrieved from Medius Weather Exchange, reported by Alabama Cooperative Extension System, Auburn University.

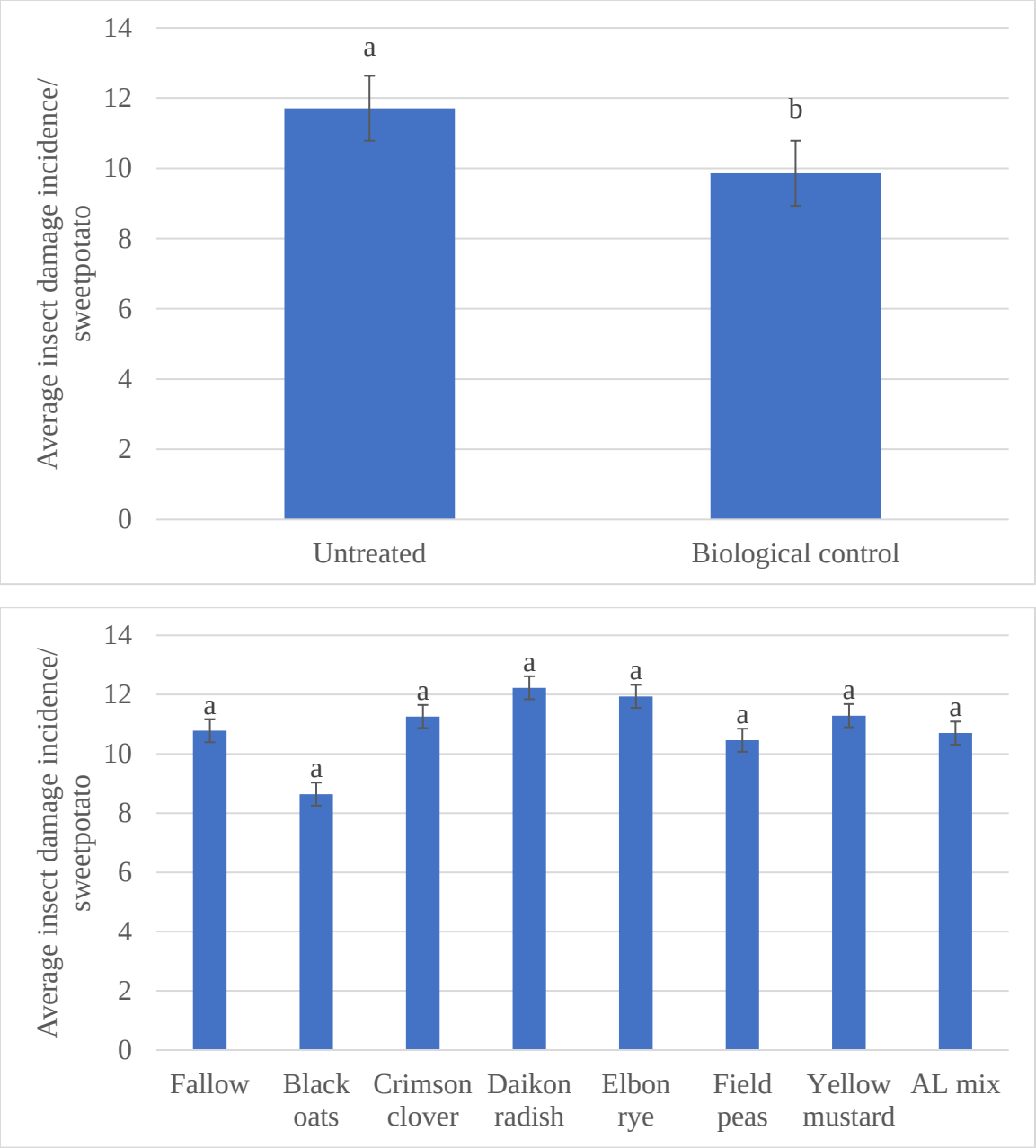
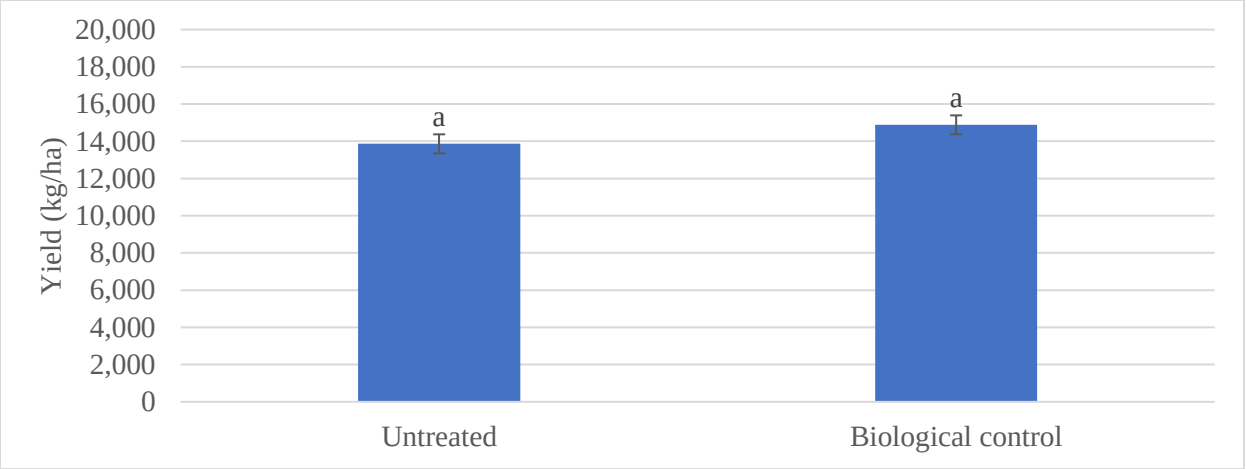


Figure 9. Effect of biological control applications and winter cover crops on the incidence of insect damage to sweetpotato roots, Alabama, 2022-2023.



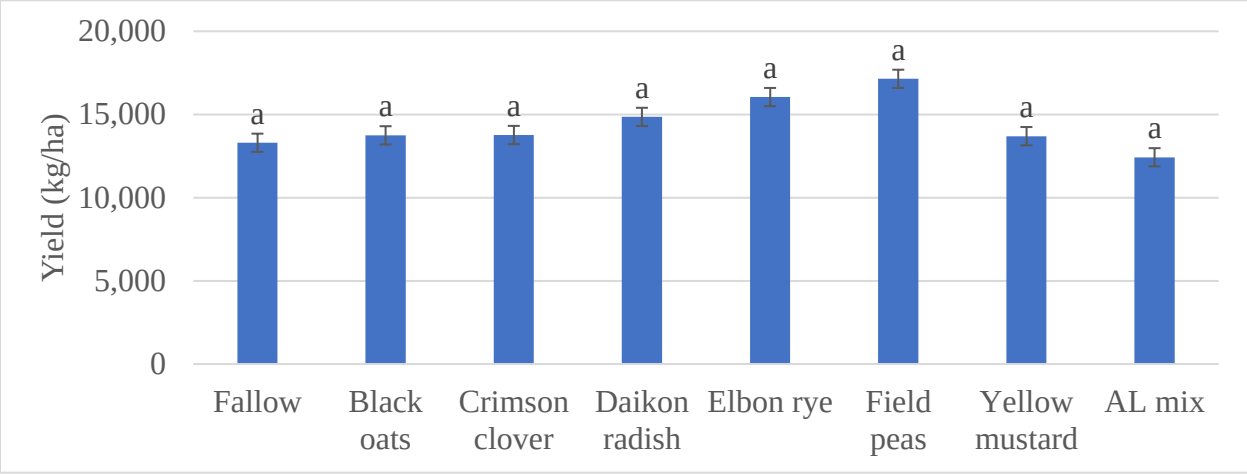
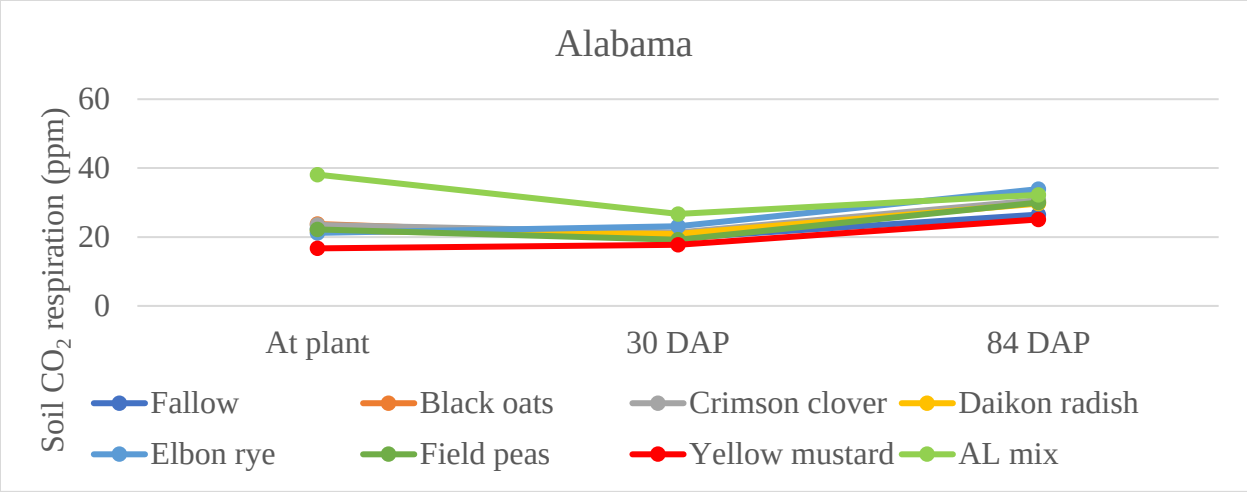


Figure 10. Effect of biological control application on sweetpotato marketable yield following the winter cover crops, Alabama, 2022-2023.



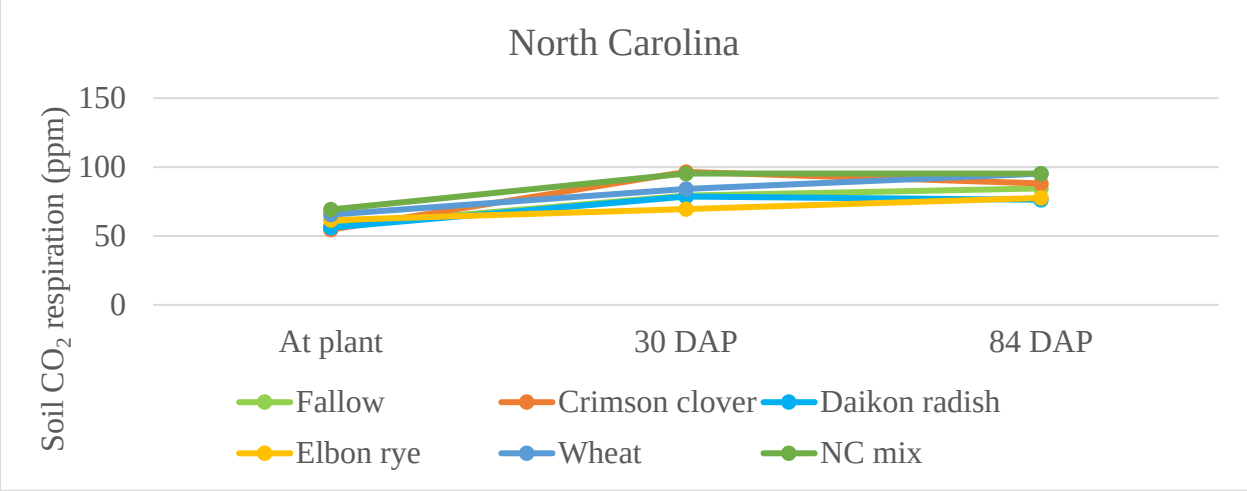


Figure 11. Effect of winter cover crops on soil CO_2 respiration during the sweetpotato cropping season measured by Solvita CO_2 Burst test in Alabama (above) and North Carolina (below) 2022-2023.

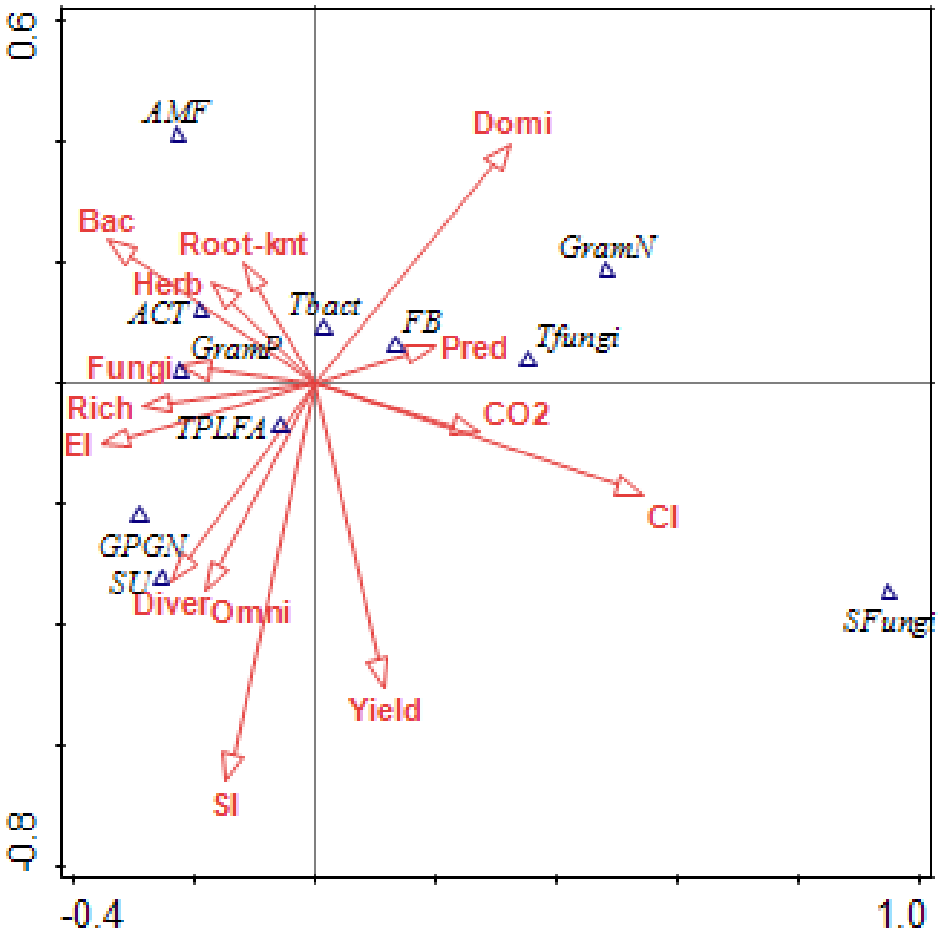


Figure 12. Canonical Analysis of Variance showing the relationships between bacterivorous nematodes (Bac), fungivorous nematodes (Fungi), herbivorous nematodes (Herb), *M. incognita* nematodes (Root-knt), predatory nematodes (Pred), Solvita CO_2 Burst measurement (CO2), nematode channel index (CI), sweetpotato yield (Yield), nematode structure index (SI), omnivorous nematodes (Omni), nematode diversity (Diver), nematode enrichment index (EI), and nematode genera richness (Rich) on the arrows and arbuscular mycorrhizal fungi (AMF), actinomycetes (ACT), gram positive bacteria (GramP, total bacteria (Tbact), fungi: bacteria ratio (FB), gram negative bacteria (GramN), total fungi (Tfungi), saprophytic fungi (SFungi), ratio of saturated to unsaturated bacteria (SU), ratio of gram positive to gram negative bacteria (GPGN), and total phospholipid fatty acids (TPLFA) on the points following the sweetpotato season in North Carolina, 2023.

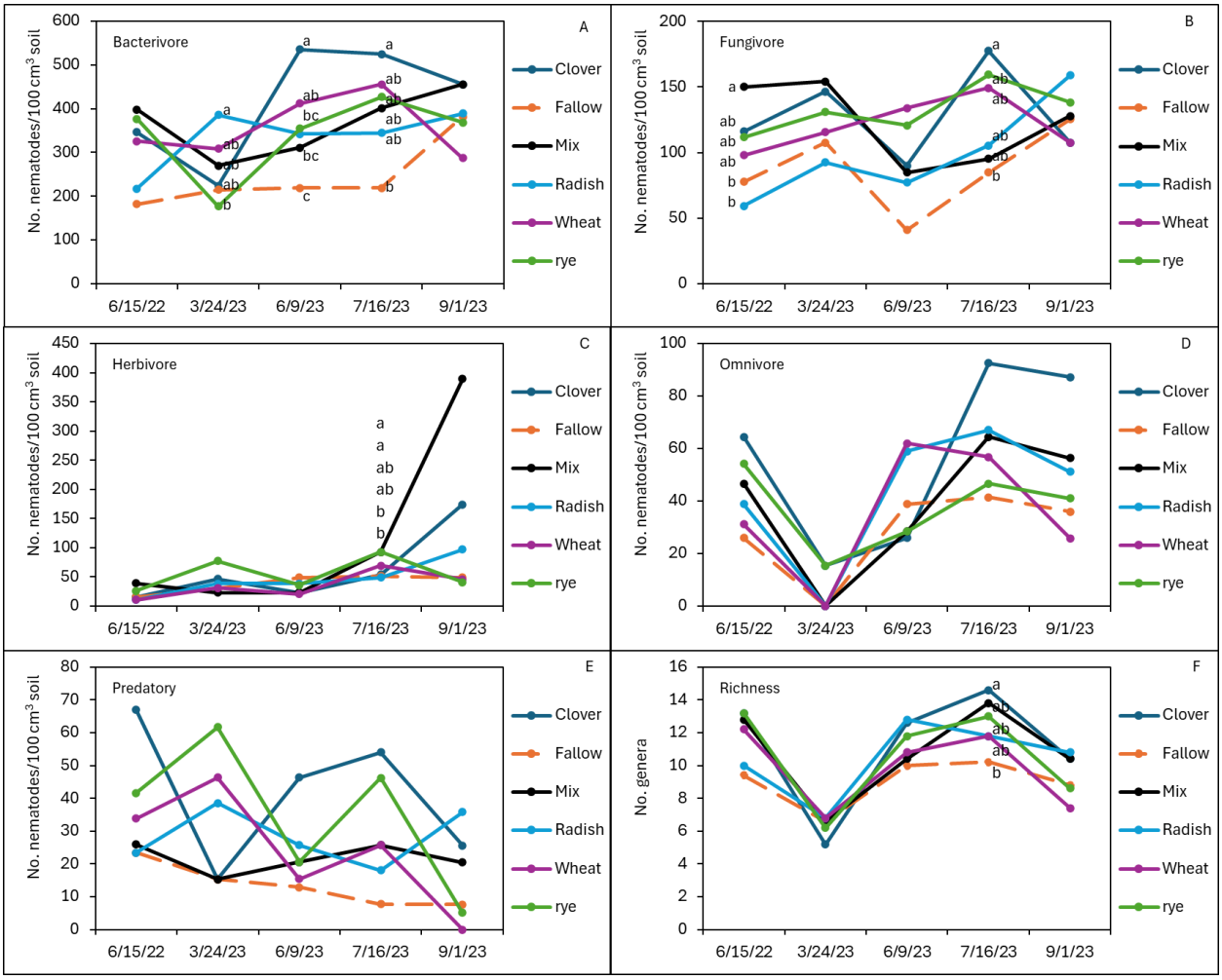


Figure 13. Soil populations of nematode trophic groups: bacterivores (A), fungivores (B), herbivores (C), omnivores (D), and predators (E), along with richness (F) during the sweetpotato growing season following the winter cover crops in North Carolina, 2023.

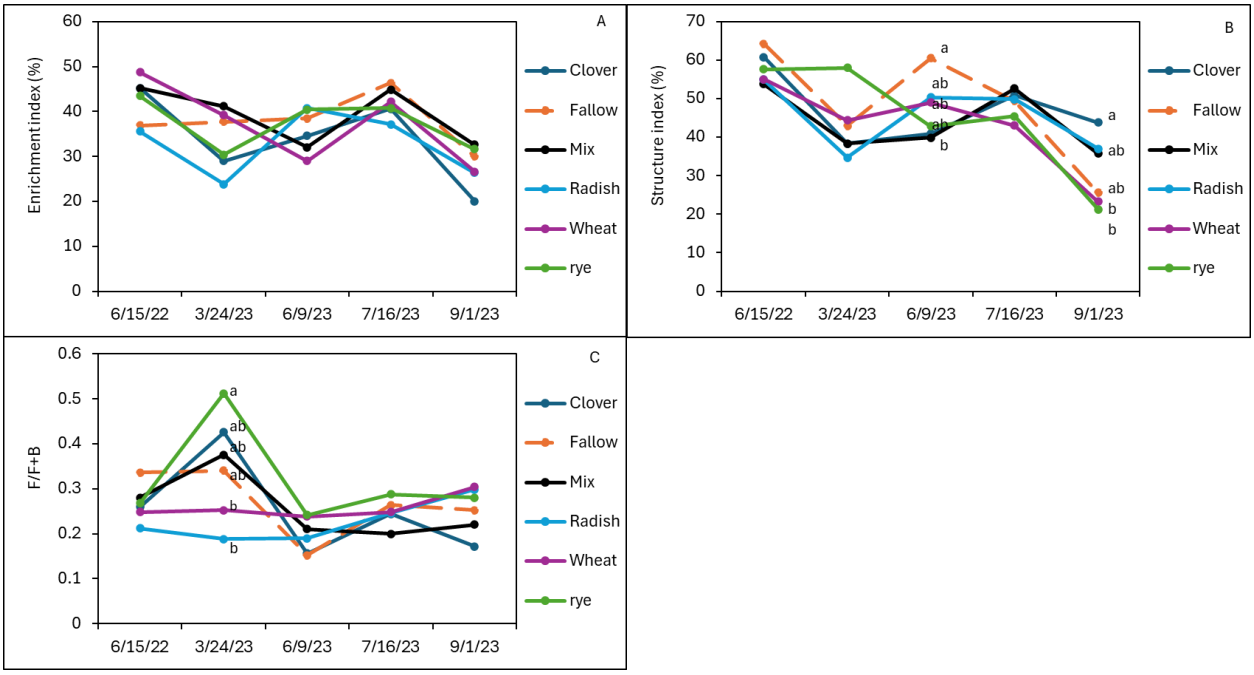
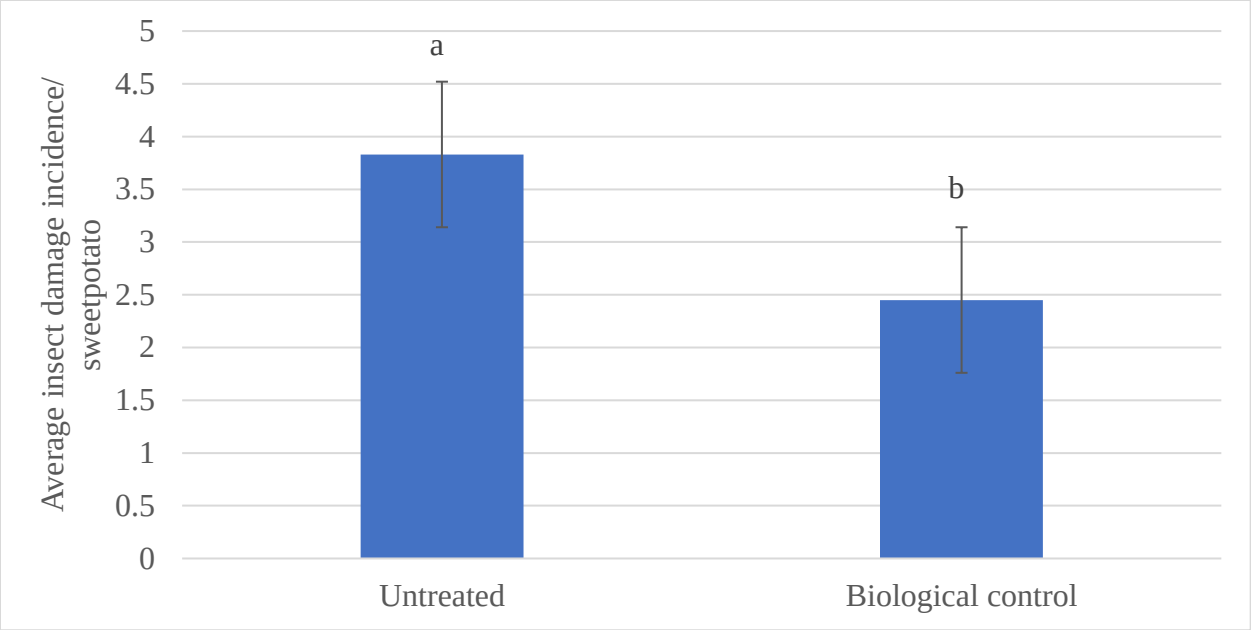


Figure 14. Soil nematode community indices of enrichment (A), structure index (B), and channel index (C) from sweetpotatoes following the winter cover crops in the North Carolina location, 2023.



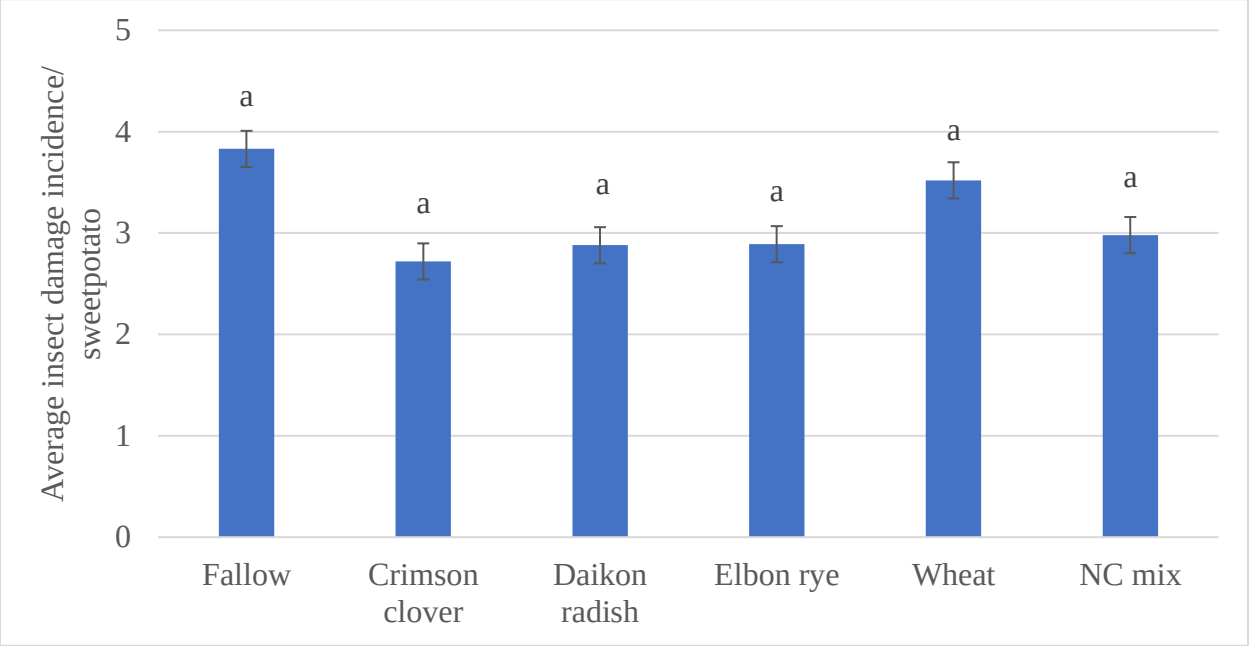
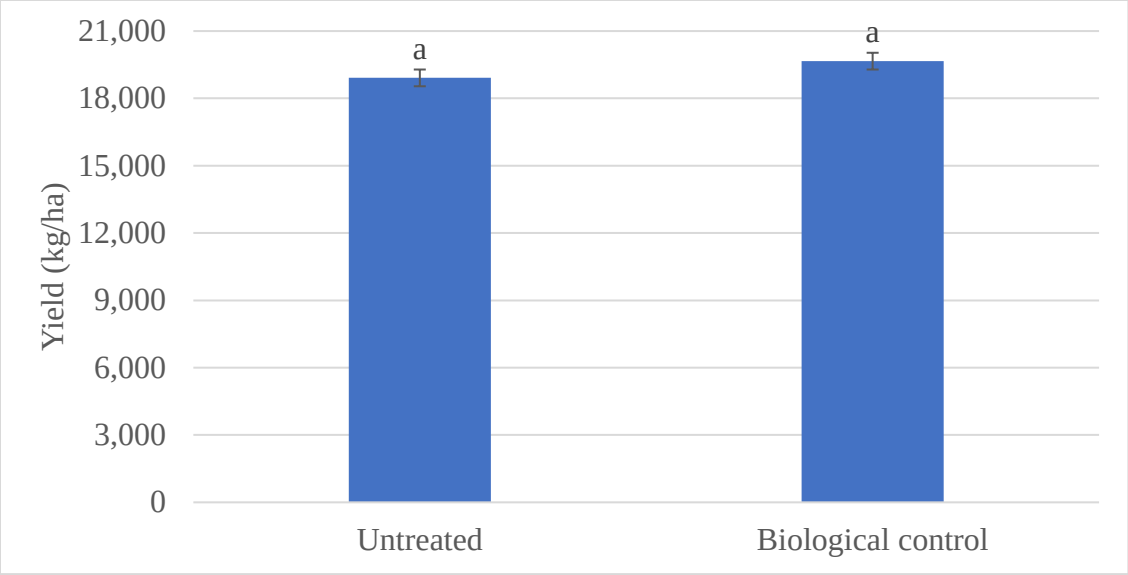


Figure 15. Effect of biological control applications and winter cover crops on the incidence of insect damage to sweetpotato root, North Carolina, 2022-2023.



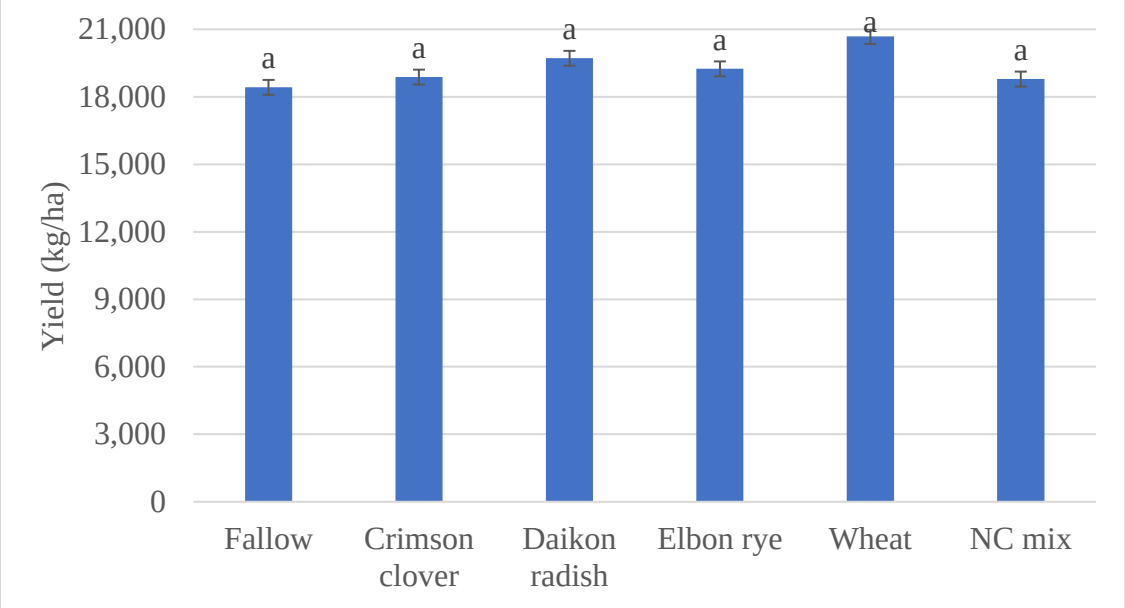


Figure 16. Effect of biological control application on sweetpotato marketable yield following the winter cover crops, North Carolina, 2022-2023.