EFFICACY, SYSTEMICITY, AND PLACEMENT OF NON-FUMIGANT NEMATICIDES FOR MANAGEMENT OF ROOT-KNOT NEMATODE IN CUCUMBER

by

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(Under the Direction of David B. Langston, Jr.)

ABSTRACT

Root-knot nematodes (RKN), *Meloidogyne* spp., are the most damaging plant-parasitic nematode to vegetable crops. Upon infection, RKN cause large galls to form on plant roots that inhibit the uptake of water and nutrients leading to yield decline. In addition, a RKN infection may predispose plants to secondary pathogens. Traditionally RKN have been managed by soil fumigation; however, the recent ban of methyl bromide coupled with increasingly stringent fumigant regulations has increased the interest in non-fumigant alternatives for nematode control. Fluensulfone is a non-fumigant nematicide that received EPA registration in 2014 for use in cucurbits and fruiting vegetables. Field trials conducted from 2011-2014 indicate that fluensulfone manages RKN when applied to soil before planting tomato or cucumber. A pre-plant incorporation or drip application of fluensulfone consistently managed RKN compared to an untreated control. Foliar applications of fluensulfone were evaluated on eggplant, tomato, squash, and cucumber to determine if systemic activity was present on these crops. Fluensulfone demonstrated systemic activity in tomato, but no other crop. Foliar applications of fluensulfone were phototoxic to cucumber and eggplant. A growth

chamber experiment and a field trial in 2012 produced evidence that a disease complex could exist between *Meloidogyne incognita* and *Pythium aphanidermatum* on cucumber. An experiment was designed and conducted twice to evaluate this potential complex. In both trials, a significant statistical synergistic interaction occurred when pots were inoculated with both pathogens than pots inoculated with either pathogen alone (P=0.015 for trial 1 and P=0.0002 for trial 2). This synergistic interaction is the first report of a disease complex involving a plant-parasitic nematode on a cucurbit crop. The adsorption-desorption and mobility of fluensulfone was evaluated on different soils. Soil organic matter and clay content significantly contribute to fluensulfone adsorption. However, these are not the only parameters that determine soil adsorption as fluensulfone adsorbed strongly to some soils with low organic matter and clay content. Mobility of fluensulfone differed among the soils tested. Fluensulfone distributed evenly through Tifton loamy sand and Greenville sandy clay loam, but was not evenly distributed through an Arredondo sand or a Chualar sandy loam.

Key words: *Meloidogyne*, *Pythium,* fluensulfone, nematicide, methyl bromide, nematode, adsorption, pesticide mobility, systemic activity

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DEDICATION

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Background and Reasons for Undertaking Work

Root-knot nematode (RKN), *Meloidogyne* spp., is the most destructive genus of plant-pathogenic nematode in the world and an important pathogen on cucumber (*Cucumis sativus)* and numerous of other vegetable and row crops. Once a cucumber plant is infected by a RKN, roots of the plant will typically exhibit galls and appear misshapen. Chlorosis and reduction of plant vigor and yield are common symptoms of RKN infection due to a decrease in nutrient and water uptake by the plant. In severe RKN cases plant death may occur. RKN also may predispose the plant to secondary infections by other soil borne pathogens. It has been noted in field trials conducted in Tifton, Ga. that there is an increase in cucumber seedling death caused by *Pythium* spp. in plots with severe nematode galling. This potential disease complex between these two organisms has been documented in tobacco (Powell *et al*., 1971) but never in cucumber. The severity of galling is dependent upon the density of the nematode population, the susceptibility of the host, and the specific *Meloidogyne* species. In highly symptomatic crops, such as carrot, forked roots results in consumer rejection and there is zero tolerance for nematode damage. Until recent years, RKN have been controlled in vegetables through the use of fumigant nematicides (1,3-dichloropropene) and biocides

such as methyl bromide (MeBr). Production of MeBr was recently halted in 2005 due to the Montreal Protocol and today MeBr is only used through critical use exemptions (CUEs). By 2015 it is anticipated that there will no longer be any MeBr CUEs granted to states. There are other fumigant nematicides available to growers that provide sufficient control of RKN such as Telone II (1,3-dichloropropene), chloropicrin, and dimethyl disulfide (DMDS, Paladin). However, as with all fumigants, these products are expensive, difficult to apply, have a long re-entry period, and are under environmental scrutiny. Problems associated with fumigants underscore the need for effective non-fumigant nematicides. Fluensulfone is a non-fumigant nematicide produced by ADAMA Agricultural Solutions Ltd. Fluensulfone is in the chemical group fluoroalkenyl, is a true nematicide, and has an unknown mode of action. Preliminary studies demonstrate that fluensulfone can provide sufficient RKN control in vegetable crops. This research addresses the need to evaluate the efficacy and the appropriate application method of fluensulfone, determine the phytotoxicity to certain vegetable crops, and to evaluate the sorption potential and mobility of fluensulfone to soils. In addition to the above mentioned objectives, this research also addresses the potential disease complex between *Meloidogyne incognita* and *Pythium* spp. in cucumber.

Literature Review

Cucumber (*Cucumis sativus)* is the second most important cucurbit crop in the world with ~84.2 billion tons of the fruit produced between 2000 and 2004. (Martinez *et al*., 2006). Cucumbers produce an edible fruit that is harvested before maturity. They are consumed raw in salads or by themselves, or they can be preserved and made into pickles. Cucumbers may also be cooked and eaten. Sometimes, as a non-culinary use,

cucumbers are used as a cosmetic because of claims that they are beneficial to skin health. The nutritive value of cucumbers is negligible since they are 96% water (National Agriculture Library).

Cucumbers originated in India and are recorded being grown there as early as 1000 B.C. It is one of the plants specifically mentioned in the Bible. The cucumber reached Europe in the 9th century after spreading through Asia. Around 1539, cucumbers made their way to the United States and were cultivated by early settlers in Virginia (Pitrat, 1997; Kelley, 2009). Cucumbers thrive best in climates with average temperatures of 30^{o}C days and 20^{o}C nights. Temperatures less than 15^{o}C limit cucumber growth and temperatures below 10^{o}C can cause chilling injury or death from frost. Water demand for cucumber ranges from 40-50 cm for adequate production. Soil types that cucumbers grow best in are well drained, fertile loam soils with a pH level between 6.5 and 7.5 (Rubatzky and Yamaguchi, 1997). Coarse, sandy soils are preferred for early spring production because they tend to dry out and warm up more quickly (Kelley, 2009).

The United States ranks fifth in the world in cucumber production with 915,570 metric tons produced in 2008 (Food & Ag. Org. of U.N.). Cucumbers are grown in 11 states with Georgia being the second largest in production with 6,800 acres in 2010 with a value of 45 million dollars (Boatright and McKissick, 2010). Cucumbers are predominantly produced in the southwest portion of the state because of favorable environment and soil type.

Cucumbers may be grown on bare-ground or on plastic mulch (Kelley, 2009). The use of plastic mulch is a common practice in Georgia and the southeast for vegetable production. Cucumbers and other vegetables grown on plastic mulch are grown on a

raised bed-top which is covered with the plastic. There are several advantages to using a plasticulture system. Plastic mulches allow for earlier planting dates because heat captured by the plastic warms the soil for early maturity. Plastic mulches are also used to reduce weed pressure and help retain moisture and fertilizers. When fumigant biocides or nematicides are used, the plastic mulch helps to provide a barrier to prevent the fumigant from escaping the soil, thus increasing the efficacy of fumigant pesticides.

Drip irrigation lines are often applied under the soil and beneath the plastic. This helps to reduce splashing caused by overhead irrigation which in turn helps to prevent foliar diseases of the crop because of reduced leaf wetness. Drip irrigation lines may also be used to apply fertilizers or pesticides to the crop. Disadvantages of using a plastic mulch system include the need for specialized equipment to apply plastic, cost in removing plastic from fields, and lack of control of nutsedges (Kelley, 2010).

When growers use a plasticulture system, it is common for more than one crop to be grown on the same plastic for one or more seasons. This practice is known as double or multi cropping. A common double cropping practice in Georgia is a tomato-cucurbit rotation. In this double cropping system, tomatoes are planted in the spring during late March or early April. The crop is grown to maturity and after harvest the plants are killed with a broad spectrum herbicide such as glyphosate. A cucurbit crop such as cucumber or summer squash is then planted for the fall season. By using a double crop system growers are able to save on the input cost of buying and applying new plastic in addition to the cost that would incur from removing the old plastic from the field. There are certain pests and parasites that effect both tomato and cucurbits. One parasite in particular is the root-knot nematode (RKN) or *Meloidogyne* spp.

Meloidogyne sp.: History, Life Cycle, and Epidemiology:

Currently, there are ~100 species of *Meloidogyne* that have been described (Siddiqi, 2000). Of the many species described, four species are responsible for >90% of agricultural damage caused by RKN. These four species are *M. incognita* (Southern RKN), *M. javanica* (Javenese RKN), *M. arenaria* (Peanut RKN), and *M. hapla* (Northern RKN) (Eisenback *et al.* 1981). The southern RKN comprises 52% of infestations worldwide and is the species of focus for this research. Four races of southern RKN have been identified for management purposes. Race 1 populations are most prevalent with races 2 and 3 being less common and race 4 populations rare (Eisenback and Triantaphyllou, 1991). *Meloidogyne* spp. were economically important pests since before the first report on cucumber roots by Berkeley in 1855. Several years later *Meloidogyne* spp. were described by Müller in the late 19th century (Müller 1883). Initially Müller placed the nematode that he described into the genus *Heterodera* (Müller 1883) and it wasn't until 1949 that Chitwood revised the taxonomical status of what is now known as the *Meloidogyne* species (Chitwood 1949, Guiran and Ritter 1979). From the time that RKN's were first described by Müller, and their re-classification by Chitwood, much research has been conducted concerning the life cycle (Fig. 1.1) and epidemiology of the RKN (Stone and Smith, 1898; Atkinson, 1899; Bessy, 1911; Nagukura, 1930; Goodey, 1932; Tyler 1933a,b; Guiran and Ritter 1979). The RKNs life cycle begins as an egg with one undeveloped juvenile nematode residing inside. Eggs are laid by a mature female that is living as a sedentary endoparasite inside a host plant's root. As a sedentary endoparasite the RKN will establish a feeding site inside the root where it will remain without moving until its death. A mature *Meloidogyne* female will lay anywhere from

500 up to 1,000 eggs although some reports indicate this number can be much higher (Tyler 1933a). The eggs laid by the mature female are protected on the outside of the root by a gelatinous matrix. Eggs may also occur freely in the soil. The juvenile nematode will undergo four molts before it reaches adulthood. The first molt occurs within the egg, so when the nematode hatches it is known as a second-stage juvenile (J2). The J2 is the only stage in *Meloidogyne's* life cycle that is capable of infecting a root. The J2 will migrate through the soil to a root and infect the root just behind the root cap. Once inside the root the nematode will set up a feeding site and undergo two more molts, known as J3 and J4, as a sedentary endoparasite. Upon the fourth molt the nematode's sex is determined. If the result of the fourth molt results in a male nematode, the vermiform male will leave the root and in some species may copulate with a female. Male RKNs are non-pathogenic and usually rare within populations having many more females than males. However, it has been documented that males are found to be more common when environmental conditions are more adverse (Tyler, 1933a, Linford, 1941). If the result of the fourth molt results in a female nematode, she will remain in the root growing from a cigar shape into a fully mature, globose shaped, egg laying female. The ratio of females to males maybe extremely skewed, typically toward the female end of the spectrum. This is because some RKN species, ex: *M. hapla*, can reproduce either by parthenogenesis or amphimixis. In cases where the males are found to be rare or absent from populations, the female nematodes reproduce via parthenogenesis (Guiran and Ritter, 1979). Parthenogenesis is an asexual form of reproduction in which the males of a population are not needed in order for reproduction to occur. Two types of parthenogenesis are known among RKNs: meiotic parthenogenesis and mitotic

parthenogenesis. With meiotic parthenogenesis the chromatids of each diploid pair of chromosomes separates becoming haploid. One division stays with the nucleus while the other exists as a polar body near the cell membrane. The nuclear division will once again pair with the polar division forming a diploid haplotype. Mitotic parthenogenesis does not involve the reduction division that occurs in meiotic parthenogenesis. Instead, the diploid chromosome will replicate itself and the nucleus will separate into two daughter nuclei followed by cell division. Mitotic parthenogenesis is more common in RKN and is the only way in which *M. incognita* has been observed reproducing (Triantaphyllou, 1985). Amphimixis is sexual reproduction in which the male nematode will copulate with the female. Amphimixis is more common under adverse survival conditions when males are more plentiful. The number of life cycles that a RKN population will have throughout a year is dependent upon several factors including temperature, suitability of host in relation to its age and nutritional status, and humidity (Guiran and Ritter, 1979). Optimum temperature for reproduction of RKN varies among species. With *M. incognita,* the lowest soil temperature recorded for reproduction was found to be 10^{o}C (Vrain et al., 1978) with optimal reproductive soil temperature being 27^{o}C. At this temperature the life cycle of a southern RKN can be completed in 24 days assuming the host has sufficient nutrient capacity for nematode reproduction (Davide and Triantaphyllou, 1967; Shepperson and Jordan; 1974). RKN's thrive best in sandy soils because of the large pore space associated with these soil types (O'Bannon *et al.,* 1961, Starr *et al*., 1993, Koenning *et al*., 1996). This large pore space allows room for the nematode to swim and move more easily in the soil solution. It is these sandy soils and

favorable temperatures found throughout the Coastal Plain region of the United States and in southern Georgia that allow the RKN to be so common and cause severe damage.

Once a plant has been infected by a RKN, the infected cell and a few cells around them will become hypertrophic. These hypertrophic cells are specialized feeding cells known as "giant cells" (Bird 1961). Giant cells become multi-nucleate, contain large amounts of DNA and have a large amount of organelles (Huang et al. 1969, Jones et al. 1976). The cells around the giant cells will undergo hyperplasia or a cell number increase. It is this hyperplasia occurring around the hypertrophy that causes the galling symptoms to which RKN is known. These hypertrophic giant cells act as nutrient sinks that funnel nutrients to the parasitic nematode living inside. This in turn stresses the plant and may result in above ground symptoms such as stunting or chorosis of the leaves, which may lead to reduced yield. In addition to symptoms directly caused by RKN, damage caused by RKN may lead to secondary infections in some crops by other soil organisms such as *Fusarium* spp. in cotton (Atkinson, 1892), *Rhizoctonia* spp. in tomato (Batten and Powell. 1971), and *Pythium* spp. in tobacco (Powell et al., 1971).

Management:

Root-knot nematodes are controlled in crops by a variety of different methods including biological, cultural, host resistance, and chemical. Biological control is often achieved by several species of fungi and bacteria. Parasitism of both juvenile RKNs and RKN eggs by the fungus *Trichoderma harzianum* have proven to significantly reduce nematode populations (Windham *et al,.* 1989, Sharon *et al.*, 2001). The bacteria *Pasteuria penetrans* is an obligate parasite of RKNs and works by attaching its

endospores to the nematodes body. These endospores germinate into the nematode where the bacteria multiply and ultimately kill the infected host (Starr and Sayre 1988). Although biological controls have been proven to be effective in greenhouse studies, it has been difficult to establish populations of biological control agents in nature.

Cultural controls are environmentally friendly and use good agricultural practices such as cultivar selection and crop rotation. Using crop rotation as a means of RKN control consists of planting a non-host or very poor host crop following a susceptible crop in an effort to reduce RKN population levels. Because the RKN cannot reproduce on the non-host crop the nematode populations will decrease. The knowledge of the specific species and race of RKN that is present in a field is essential to an effective crop rotation program as some species and races may affect multiple crops grown in an area (Eisenback and Triantaphyllou, 1991). An effective crop rotation may sometimes be difficult to obtain because of multiple species in the same location. Another cultural RKN control is the use of a cover crop. Marigolds produce 5-(3-buten-1-ynyl) 2,2-bithienyl and alpha terthienyl (Morallo-Rejesus and Decena, 1982) in addition to flavonoids (Olabiyi *et al.*, 2006) that have an antagonistic effect on nematodes. The use of these cover crops during the winter has the potential to reduce nematode populations for the next growing season (Olabiyi and Oyedunmade, 2007). In addition to using good cultural controls, good sanitation practices such as washing equipment off after leaving an infested field can help to reduce the spread of nematode inoculum.

Resistance to RKN has been found in *Solanum peruvianum* (a relative of tomato) and *Capsicum* species (pepper). The gene responsible for the resistance in *Solanum* sp. is the *Mi* gene, and this gene has been bred into many tomato cultivars currently available

today (Smith 1944). The *Mi* gene confers resistance to a broad spectrum of species of *Meloidogyne;* however, this resistance has been documented to be broken at soil temperatures above 28^{o}C (Dropkin, 1969) and has also been documented to be broken in the field by virulent populations of nematodes (Roberts *et al.*, 1990; Castagnone-Sereno *et al.*, 1996; Kaloshian *et al.*, 1996). At least 5 resistance genes (*Me 1-5*) have been found in pepper with two of the genes (*Me 1* and *3*) conferring the same broad resistance as the *Mi* gene (Castagnone-Sereno *et al*. 2001). Both the *Mi* gene and the *Me* genes elicit a hypersensitive response (Paulson *et al*. 1972, Bleve-Zacheo *et al*. 1998) resulting in host cell death by recognizing nematode feeding thus preventing the nematode from establishing a feeding site and gall formation. Resistance to RKN in cucumber has been reported by Walters *et al.* (1993); however, currently there are no available commercial cucumber varieties that demonstrate the resistance shown in these studies. Trials have been conducted using resistant tomato and pepper cultivars to reduce nematode pressure on second crop cucurbits. Colyer *et al.* 1998, demonstrated that RKN resistant tomato cultivar 'Celebrity' significantly reduced root gall ratings on second crop cucumber compared to cucumber following a susceptible cultivar, 'Heatwave', and an ethoprop nematicide treatment. Similar results were found when RKN resistant bell pepper variety 'Charleston Belle' was used to significantly reduce gall ratings on second crop cucumber (Theis *et al.* 2004).

Nematicides have been used extensively in high value crops for control of nematodes throughout most of the last century. Nematicides may be grouped into two separate categories: those that kill nematodes (nematicides) and those that temporarily paralyze nematodes (nematistats). Nematicides are mostly fumigants that are injected

into the soil. Fumigant nematicides are generally liquid formulations that volatilize upon soil entry. Available fumigants may be lumped into two broad categories: halogenated hydrocarbons and those that discharge carbon disulfide or methyl isothiocyanate (Nyczepir and Thomas, 2009). Fumigants may be further categorized as either nematicidal (kills only nematodes) or multi-purpose (kills nematodes, weeds, insects, fungi) (Lembright, 1990; Dunn and Noling, 1997). Chemical control of RKN in cucumber has relied heavily upon the application of the multi-purpose soil fumigant methyl bromide (MeBr) (Christie and Cobb, 1940) since the middle of the last century, especially in cucumbers that are grown on plastic mulch. However, importation and manufacturing of MeBr was phased out beginning January 1, 2005 because MeBr was found to be an ozone depleting substance. MeBr is only used today through critical use exemptions (CUEs) with no CUEs being granted to states by 2015. MeBr is an example of a halogenated hydrocarbon fumigant. There are other halogenated hydrocarbons that are still available to growers today including: chloropicrin and 1,3 dichloropropene (Telone II). Chloropicrin is used as a multi-purpose fumigant while Telone II has more targeted nematicidal properties. Metam sodium (Vapam) is a liquid fumigant that releases methyl isothiocyanate upon its entry into the soil. This compound is not very volatile, therefore it is dependent upon soil moisture to reach the target pest. Although these fumigants do offer favorable control of RKN they are difficult to apply, costly to the grower, have a long re-entry period, and are under the scrutiny of certain environmental agencies due to their volatility and toxicity. Because of these problems associated with fumigant pesticides, growers desire an effective non-fumigant nematicide.

Previously, non-fumigant nematicides have played an important role in nematode control options. Current non-fumigant nematicides are either organophosphates or carbamates; both are nematistats and both are either formulated as granules or liquids with both chemical classes acting as acetylcholinesterase inhibitors (Opperman and Chang, 1990; Haydock et al., 2006). Nematistats paralyze a nematode as long as the active ingredient is present in strong enough concentrations in the soil solution. However, once concentrations of the nematistats fall below activity levels, the nematode is able to recover and become infectious again. In order for nematistats to provide sufficient control, they must paralyze the nematode long enough for the crop to become established (4-8 weeks) so that a nematode infection would have little impact (Wright, 1981a; Rich *et al*., 2004). Application methods of non-fumigant nematicides can vary depending on the product that is being applied. Many organophosphates and carbamates are formulated as both granules and liquids. Granule nematicides are often applied pre-plant to a bed top and then incorporated into the soil 2-4 inches prior to planting. Liquid formulations may also be applied pre-plant where the nematicide is sprayed on a bed surface and then incorporated into the soil prior to planting. However, liquid formulations may also be applied as a foliar spray, drench, or applied through a drip irrigation. Oxamyl is recognized as a non-fumigant nematicide with basipetal and acropetal systemic activity and is one of only two non-fumigant nematicides labeled for control of RKN on cucumber in Georgia (Georgia Pest Management Handbook, 2013). Foliar and root applications of oxamyl have been shown to arrest nematode development however, nematodes recover from oxamyl treatments and further develop into adults once oxamyl concentrations declined (Wright and Womak, 1981b). Oxamyl is commonly

used in combination with fumigant nematicides as a post-plant treatment throughout the growing season. Oxamyl does not provide sufficient control of RKN when applied as the only nematicide treatment but yield increases have been observed when oxamyl is used in combination with a soil fumigant (Desaeger *et al.*, 2004). This could be due to the plant growth stimulating effect of carbamate pesticides. Ethoprop, or Mocap (Bayer CropScience), is also labeled in Georgia for control of RKN in cucumber (Georgia Pest Management Handbook, 2013). Ethoprop is formulated as both a granular and an emulsifiable concentrate. Like oxamyl, ethoprop provides the most favorable nematode control when used in combination with a soil fumigant (Pinkerton *et al.*, 1986). Biodegradation can become a factor in the efficacy of a non-fumigant nematicide because multiple applications, that may be made in a single season, can be conducive to the buildup of soil microorganisms that can degrade the chemical (Davis *et al.* 1993; Smelt *et al.*, 1996; Lawrence *et al.*, 2005). With this limited number of non-fumigant nematicides available to growers today along with the problems that are associated with fumigant nematicides, a need has arisen for a new non-fumigant nematicide for control of RKN in cucumber.

The sorption of pesticides to soil particles reduces efficacy against target species when bound, making them unavailable in the soil solution. This is an important concept where contact nematicides are concerned because the target species are within the soil solution; therefore, effective nematicide concentrations must be soil solution for adequate control (Smelt and Leistra, 1992). In addition to reducing efficacy, pesticide adsorption affects mobility throughout the soil profile, which can impact its influence on the environment (Karpouzas *et al.*, 2007). The sorption of other non-fumigant nematicides

has been considerably researched with many studies finding a strong correlation between sorption and soil properties (Pantelelis *et al.,* 2006; Qin *et al*, 2004; Simon *et al*., 1992; Bilkert and Roa, 1985; Gerstl, 1984). The sorption of fluensulfone to soils has not been examined.

Fluensulfone, or NIMITZ, is a non-fumigant nematicide in the fluoroalkenyl chemical group that is scheduled for registration for use in cucurbit and solanaceous crops in 2014 and is being produced by ADAMA Agricultural Solutions Ltd. It is a true nematicide with an unknown mode of action that is being formulated as an emulsifiable concentrate with 480 grams of active ingredient per liter (MANA). Preliminary studies with this compound show promising results for control of RKN in various crops (Oka *et al.* 2009; Csinos *et al*. 2010) with some studies indicating that this compound may have systemic activity in the plant for control of RKN (Oka *et al.* 2012).

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Figures

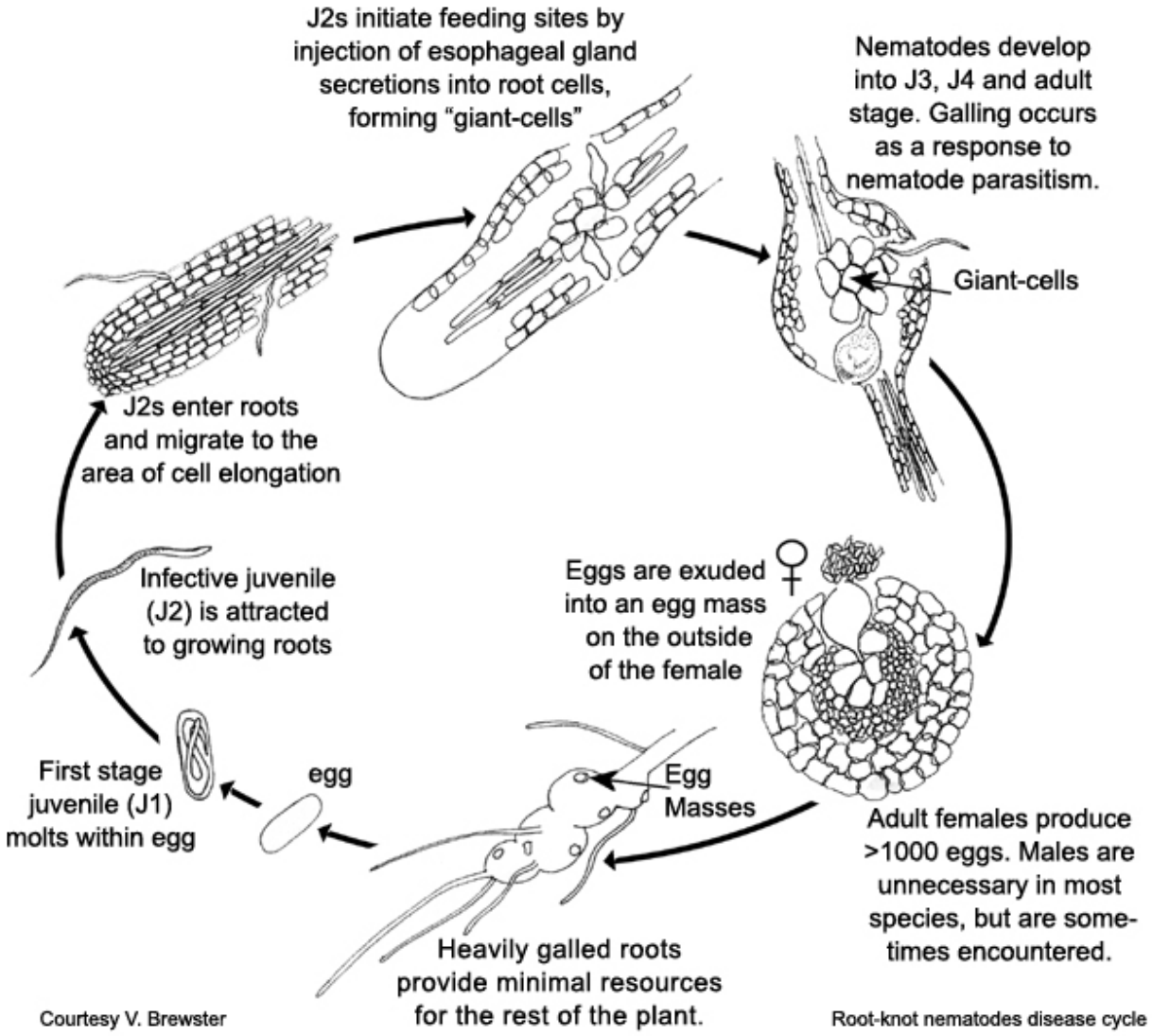

Figure 1.1 Life cycle of a root-knot nematode.

CHAPTER 2

EFFICACY OF FLUENSULFONE USING PRE-PLANT INCORPORATION AND DRIP APPLICATION METHODS

[1]Morris, K.A., D.B. Langston, R.F. Davis, P. Timper, J.P. Noe, and D.W. Dickson. To be submitted to *Pest Management Science.*

Abstract

Fluensulfone is a new nematicide in the flouroalkenyl chemical group. Four field trials were conducted in 2012 and 2013 to evaluate the efficacy of different application methods of fluensulfone for control of *Meloidogyne incognita* in cucumber (*Cucumis sativus*). Treatments of fluensulfone were applied either pre-plant incorporated (PPI) or via three different drip irrigation applications. All fluensulfone treatments were applied at a rate of 3.0 kg a.i./ha. Drip treatments were separated by using different pulse irrigation methods, and included: no pulse irrigation, pulse irrigation 1 hr after treatment, and treatment during the first cycle of pulse irrigation. A PPI treatment of oxamyl at a rate of 22.5 kg a.i./ha was used in the trial as an industry standard. Plant vigor, harvest data, and root gall ratings were recorded. In the spring of 2012, all drip treatments of fluensulfone improved vigor ($P \leq 0.05$) and decreased gall ratings when compared to the oxamyl treatment and the untreated check ($P \leq 0.10$), and improved yield when compared to the untreated check ($P \leq 0.05$). Stand counts were recorded in the fall of 2012 because of high seedling mortality caused by *Pythium* spp. All nematicide treatments had higher stand counts compared to the untreated check ($P \leq 0.05$). Also in the fall of 2012, fluensulfone PPI and treatment during the first cycle of irrigation yielded better than either oxamyl or the untreated check ($P \leq 0.05$). In 2013, none of the drip applications of fluensulfone improved plant vigor, final yield, or reduced root galling compared to the untreated control ($P \leq 0.10$). The incidence of nematode galling was reduced in the spring of 2013 for drip application without a pulse and when pulse irrigation was applied 1 hr after treatment ($P \leq 0.10$). These data suggest that control of root-knot nematode may be obtained with pre-plant drip injections of fluensulfone.

Introduction

Southern root-knot nematode (RKN), *Meloidogyne incognita* (Chitwood) is one of the most economically important plant-pathogenic nematodes that affect cucumbers. The pathogen enters the root and establishes a feeding site which results in large gall formation in a susceptible host. These large galls impair the ability of the plants to uptake water and nutrients which can lead to symptoms such as wilting, stunting, chlorosis and ultimately yield loss (Karssen *et al.* 2013). In addition, an infection by RKN may also predispose a plant to secondary pathogens (Batten and Powell, 1971; Atkinson, 1892).

Many vegetable crops are grown in a plasticulture system and consequently RKNs have traditionally been controlled through the use of fumigant nematicides and biocides such as methyl bromide (MeBr), 1,3-dichloropropene (Telone® II), chloropicrin, or a mixture of these compounds. The plastic mulch is applied over the top of the fumigated soil to slow the dissipation of the highly volatile fumigant and prevent it from escaping the treated area thereby increasing the efficacy of the compound. Fumigant nematicides can be highly efficacious against nematodes; however, they are costly, require specialized application equipment and buffer zones, are highly volatile, present worker safety concerns, are not environmentally friendly, and have a long period of time between treatment and planting date (plant-back interval) due to phytotoxicity concerns. As of 2005, MeBr was banned via the Montreal Protocol and its use was discontinued in 2014 except in certain situations where it may still be applied through the use of critical use exemptions (CUEs).

Currently there are several non-fumigant nematicides that are available to growers. These products are primarily organophosphates or carbamates, both of which pose significant worker safety concerns because they are acetyl cholinesterase inhibitors that are highly toxic to humans. In addition to toxicity concerns, both of these chemical classes are readily leached through soils, which pose a risk for ground water contamination (Haydock *et al*., 2013). Furthermore, organophosphates and carbamates are not true nematicides but rather are nematistats. Nematistats temporarily paralyze nematodes but once the level of active ingredient falls below a pernicious level the nematodes can recover (Rich *et al.*, 2004; Wright, 1981).

Fluensulfone is a non-fumigant nematicide that has recently received an Environmental Protection Agency (EPA) registration and is now for sale in the U.S. Fluensulfone is in the flouroalkenyl chemical class and has an unknown mode of action (Kearn *et al.*, 2014). Fluensulfone is also a true nematicide, which kills nematodes once they come in contact with the active ingredient (Oka *et al.*, 2009). Unlike fumigant nematicides, fluensulfone is a water soluble compound and moves through the soil water. Fluensulfone has a lower mammalian toxicity (LD_{50}> 500 mg/kg) than organophosphates and carbamates, which allows for safer application. Fluensulfone is being produced by ADAMA Agricultural Solutions Ltd. and will be sold in the U.S. under the trade name NIMITZ™. The initial label allows for the product to be applied to the fruiting vegetables and cucurbits.

The published literature contains limited data on the field efficacy of fluensulfone. However, some preliminary studies have demonstrated positive results with fluensulfone for control of RKN (Oka *et al.*, 2012; Csinos *et al.*, 2010). The

objective of these trials was to evaluate the efficacy of fluensulfone when applied by various application methods. Fluensulfone was evaluated for control of RKN by pre-plant incorporation (PPI) and three drip application methods.

Materials and Methods

Site Description and Land Preparation. Four field trials were conducted at the University of Georgia Coastal Plains Experiment Station during the summer and fall of 2012 and 2013. All trials were at Hort Hill Farm Tifton, GA but each trial was at a different location on the farm each year. All trials were conducted on a Tifton sandy loam soil type (83.6% sand, 10.2% silt, and 6.2% clay). The area has a history of vegetable crops and a natural infestation of *Meloidogyne incognita* race 1.

In 2012, the area was harrowed and roto-tilled 18 May for the spring trial and 23 August for the fall trial. Plots were 4.6 m long and 0.8 m wide with a 3 m alley between plot ends. Beds were shaped and low density polyethylene plastic film was laid on 21 May and 27 August using a commercial tractor-drawn bed shaper.

In 2013 the area was harrowed and roto-tilled on 27 May for the spring trial and 30 July for the fall trial. Plots were the same dimensions as described for the previous year. Beds were shaped with a tractor drawn bed shaper and white plastic mulch was laid on 29 May and 5 August for the spring and fall, respectively. Each test utilized a randomized complete block design with eight replications per treatment.

General Management: John Deere T-Tape® with 6 mm walls and 15.54 cm emitter spacing was used in the 2012 spring trial. All other trials used a 6 mm Chapin® Drip-Tape with 15.24 cm emitter spacing. One drip tape line per bed was applied 2-4 cm

underneath the white plastic mulch. Two-week old cucumber seedlings were purchased from Lewis Taylor Farms, Tifton, GA and used in all trials. In all trials, 15 cucumber seedlings were planted per plot on 30.5 cm spacing. In 2012, 'Impact' cucumbers were planted in the spring trial on 11 June and 'Diomede' cucumbers were transplanted on 7 September in the fall trial. In 2013, 'Diomede' was initially planted in the spring trial on 7 June; however, due to high seedling mortality these plants were re-planted with '3462' on 18 June. 'Impact' cucumbers were transplanted on 15 August 2013 for the fall trial.

All trials received a row middle herbicide tank mix application of paraquat (0.92 L/ha), flumioxazin (217.85 ml/ha), and ethalflurilin (0.61 L/ha) to provide season long weed control on 23 May, 4 September, 29 May, and 5 August for 2012 spring and fall and 2013 spring and fall, respectively. In addition to pre-plant herbicide sprays, halosulfuron (72.6 ml/ha) was applied post-transplant to all trials once plants had reached the 2-4 true leaf stage for control of purple and yellow nutsedge. Foliar and soilborne diseases, and insects were controlled using University of Georgia Extension recommendations.

Fertilization: Granulated (10-10-10) with minor nutrients (0.06% B, 9% Cl, 0.06% Cu, 0.36% Fe, 0.15% Mn, and 0.14% Zn) was applied and roto-tilled into the soil at a rate of 1,120 kg/ha before bed formation. Liquid fertilizer (7-0-7) with 2% Ca. and 10% S was applied following University of Georgia Extension recommendations weekly beginning 2 weeks post-transplant until harvest using a CO_2 pressurized stainless steel tank attached to the drip irrigation system.

Treatments: Fluensulfone (treatment A) at 3.0 kg a.i./ha and oxamyl (DuPont Crop Protection) (treatment E) at 22.5 kg a.i./ha were applied pre-plant and incorporated

on 21 May and 27 August 2012 and 29 May and 5 August for 2013. PPI applications were All incorporated treatments were applied using a CO_2 pressurized backpack sprayer with a 4 nozzle boom calibrated to deliver 187 L/ha with 8002VS tips. A reduced rate of fluensulfone (41.6 ml) was mixed in a 3 L bottle and applied to plots. The spray treatment was applied to a soil area 1.8 m wide and then a bed-top was formed of 0.75 m. This treatment was incorporated into the soil immediately after application with a PTO-driven roto-tiller. Drip applications of fluensulfone were made on 1 June and 4 September 2012 and 4 June and 8 August 2013. All injected treatments were applied at a rate of 3.0 kg a.i/ha using a CO_2 pressurized bottle attached to a manifold system that allowed injection of nematicide to be made to each drip irrigation treatment. Injected treatments were mixed in a 3 L bottle at a rate of 17.4 ml/6 plots. For both trials in 2012, all plots were irrigated 24 hr before nematicide treatment to ensure that soil was at field capacity throughout the bed. Volumetric water content (VWC) readings were recorded at the time of injections using a soil moisture sensor (10HS soil moisture sensors, Decagon, Pullman, Wa.) in m^3/m^3. Three sensors were placed 13 cm below the soil line in one bed receiving each injection treatment. One sensor was placed in the center of the bed, another 18 cm from the center of the bed, and another on the bed shoulder. In 2012, VWCs at time of injection were 26% (middle), 27% (17.78 cm), and 28% (shoulder) for the spring trial and VWCs for the fall trial were 32% (middle), 24% (17.58 cm), and 22% (shoulder). In 2013, only drip application treatments received the 24 hr irrigation before injection. In 2013, VWC readings at time of injection were 29% (middle), 28% (17.57 cm), and 27% (shoulder) for the spring and moisture readings for the fall trial were 27%

(middle), 27% (17.57 cm); the third sensor malfunctioned so no VWC data was collected for the shoulder of beds in the 2013 fall trial.

Drip nematicide treatments either received no subsequent pulse application, pulse irrigation 1hr after nematicide injection, or nematicide was applied at the beginning of the pulse irrigation. Pulse irrigation consists of a water event being turned on and then off for a period of time before then being turned back on. Pulse irrigation is used in an effort to conserve the amount of water used during an irrigation event (Karmeli and Peri, 1974). All drip treatments were allowed a 15 min priming period before injection. The drip treatment which received no subsequent pulse application was applied after the initial 15 min irrigation event. Irrigation was applied to these plots for an additional 15 min to ensure that the nematicide was flushed from the lines. Pulse treatments for these trials started 1hr after the initial 15 min injection cycle had ended. Water was turned on for 15 min and off for 15 min and then on again for 15 min for a total of 4 cycles (Table 2.1)

Data Collection: In all trials, soil cores were collected from the middle of plots before treatment application and then again after root gall ratings to assess RKN densities before and after treatment. Five soil cores were taken from the middle of each plot using 10.16 x 30.5 cm conical sampling probes. Samples were sent to the University of Georgia Nematology Lab (Athens, GA) and RKN juveniles were counted per 100 cm^3 of soil.

Vigor ratings were conducted in all trials to evaluate the effect of treatment on above-ground plant parts. Vigor ratings were evaluated on a 0 to10 scale with 0 being a dead plant and 10 being live, vigorous, healthy plant. In 2012, vigor ratings were recorded 14, 21, and 29 days after planting (DAP) for the spring trial and 14 and 26 DAP

for the fall trial. In 2013, vigor ratings were recorded 14 and 21 DAP for the spring trial and 14 and 19 DAP for the fall trial. Stand counts were recorded 17 DAP for the fall trial (2012) and 19 DAP for the fall trial (2013) due to high seedling mortality from *Pythium* spp. in addition to plants being eaten by deer.

In 2012, cucumbers were harvested once a week for three consecutive weeks on 20 July, 26 July, and 2 August for the spring trial and 24 October, 1 November, and 6 November for the fall trial. In 2013, cucumbers were harvested once a week for three consecutive weeks on 22 July, 26 July, and 30 July for the spring trial and 24 September, 1 October, and 8 October for the fall trial. All marketable fruit was harvested and fruit weights and fruit counts were recorded for each plot.

Root gall ratings and nematode incidence per plot were recorded after the last harvest on 2 August and 6 November in 2012 and on 30 July and 9 October in 2013. Seven plants were exhumed from each plot to serve as the sample for gall ratings and incidence. Not all plots in the fall trial (2012) had seven live plants at the time of gall ratings so all live plants were recorded from these plots. If there were found to be no living plants in a plot, plots were dropped from nematode assessment. Root-gall ratings were measured on a 0 to 10 scale with 0 being no visible galls and 10 being 100% of root system infected with galls. Nematode incidence was measured by the percentage of plants exhumed that were galled.

Statistical Analysis: All data was subjected to analysis of variance using ARM data management software (Gylling Data Management, Brookings, SD). Statistical comparisons were made using Fisher's protected LSD test ($P \leq 0.05$) for nematode

counts, vigor, stand count, and yield data. Statistical comparisons were made using Fisher's protected LSD test ($P \leq 0.10$) for gall ratings and pest incidence data.

Results

Nematode enumeration: Nematode counts were transformed using $\log_{10}(x + 1)$ to normalize the data and then back transformed using 10^x to represent the number of J2s per plot. There was a reduction in the number of J2s found in the soil after harvest in the spring 2012 trial for the pulse irrigation 1 hr after treatment and treatment during the first cycle of pulse irrigation ($P \leq 0.05$). There was also a significant difference in the number of J2s found in the soil during the spring of 2013 prior to treatment application ($P \leq 0.05$). No differences were noted in any other trial in either year. All nematode enumeration data is summarized in Tables 2.2 and 2.3.

Effect of nematicides on plant vigor and stand counts: In the spring of 2012 all fluensulfone drip applications demonstrated greater vigor for all 3 rating dates than the fluensulfone PPI treatment or the untreated check (Table 2.4). The drip applications also showed vigor improvement over the oxamyl treatments for 2 of the 3 rating dates. During the 2012 fall trial all treatments had greater vigor ratings during the first rating date with the exception of pulse irrigation 1 hr after treatment ($P \leq 0.05$). There was no statistical separation among treatments for the second vigor ratings. Vigor ratings for 2013 field trials differed from the data collected in 2012. In the spring of 2013, the vigor rating for pulse irrigation applied 1 hr after treatment was less than the untreated control for the first rating date. No pulse irrigation and pulse irrigation applied 1 hr after treatment were less than the untreated control for the second rating date. There were no statistical differences in vigor observed between the untreated control and the PPI

treatment of oxamyl or fluensulfone. The fall of 2013 showed similar results to the spring of 2013 with pulse irrigation applied 1 hr after treatment being significantly lower than the fluensulfone PPI treatment or the oxamyl treatment. Drip applications of fluensulfone were not different from the untreated check in the fall of 2013 with the exception of the no pulse irrigation treatment for the second rating date.

Stand counts were recorded in the fall of both years because of high seedling mortality. In both 2012 and 2013, all fluensulfone treatments had significantly greater stand counts than the untreated control (Table 2.5).

Effect of nematicide treatments on yield: In the spring of 2012, all fluensulfone drip application treatments demonstrated greater yield than the untreated check. Fluensulfone and oxamyl applied pre-plant incorporated did not increase yield when compared to an untreated control in this trial (Fig. 2.1a). During the 2012 fall trial only, the fluensulfone PPI treatment and the treatment applied during the first pulse cycle improved yield over the untreated check or any other nematicide treatment (Fig. 2.1b). In this trial the untreated control yielded a total of 0 pounds of fruit for the season. During the spring of 2013, no nematicide treatment produced greater yield than the untreated control (Fig. 2.2a), probably due to low nematode populations in this trial. Pre-plant incorporations of fluensulfone and oxamyl showed significantly greater yield than the untreated check or any of the fluensulfone drip applications during the fall of 2013 (Fig. 2.2b)

Effect of nematicide treatment on root galling: All fluensulfone drip applications reduced gall ratings compared to the untreated check and either of the PPI treatments during the spring of 2012 (Fig. 2.3a). There were no statistical differences among

treatments during the fall of 2012 or the spring of 2013 (Table 2.6). During the fall of 2013 fluensulfone applied as a PPI and applied during the first pulse cycle significantly reduced root galling compared to any other nematicide treatment or the untreated check (Fig 2.3b). Fluensulfone significantly reduced nematode galling incidence in the spring trials of both years compared to an untreated control (Table 2.7).

Discussion

In these trials, the efficacy of fluensulfone was variable across trials and years making it difficult to determine the most effective application method. Since this nematicide is a water soluble compound, the 24 hr irrigation event in 2012 may have diluted fluensulfone concentrations in the PPI treatments to a level that decreased its efficacy. Like the carbamates and organophosphates, fluensulfone is a non-fumigant nematicide and therefore there is a risk of losing the active ingredient due to excessive rainfall or irrigation (Noling, 2011; Rich *et al.*, 2004; Apt and Caswell, 1988; Schneider *et al*., 1988; Brodie, 1971). The inconsistent efficacy of the drip applications could be due to initial inoculum levels in the soil before application. Drip applications did not control RKN in the fall of 2012 and in the spring of 2013 compared to an untreated control. The average number of J2s per 100 cm^3 of soil before treatment was very high in the fall 2012 (464) and very low in the spring of 2013 (1). In contrast, the spring of 2012 and the fall of 2013 had only moderate numbers of J2s per 100 cm^3 of soil (120 and 137, respectively) and in both of these trials drip applications of fluensulfone reduced root galling. These results indicate that there could be a threshold level of initial inoculum that could influence whether a fluensulfone application would be beneficial. In high pressure situations, there may be enough inoculum in the soil to leave enough J2s after

treatment to cause damage, and in low pressure situations the lack of inoculum would not warrant a nematicide application. Additional studies are needed to document such a threshold.

The reduction in plant vigor following a fluensulfone application indicates that crop injury may occur with this product if it is not sufficiently washed from the bed prior to planting or if the plant-back interval is too short. Label recommendations for fluensulfone state that a 7-day plant-back interval is necessary for drip applications and a 10-day plant-back interval for PPI applications to avoid crop injury. In 2012 there was no reduction in plant vigor observed and the plant-back intervals for drip applications were 11 days and 3 days for the spring and fall, respectively. In 2013, a significant reduction in plant vigor was noted with all drip applications. The plant back intervals for these trials were 3 days in the spring and 7 days in the fall. It is unclear why phytotoxicity was not observed in the fall of 2012 with such a short plant-back interval. It is possible that an additional irrigation event after treatment but prior to planting could have mitigated crop injury from fluensulfone in 2012. Currently, fumigants are the primary means of nematode control in the southeastern U.S. In many cases combinations of different fumigants are used to increase efficacy against a broad spectrum of pests. Crop injury can be a major concern when fumigants are used, especially in plasticulture systems and therefore they require long plant-back intervals. Csinos *et al.* (2002), reports that a plant-back interval of 36 days was necessary to achieve optimum stand counts when a combination of 1,3-dichloropropene, chloropicrin, and metham sodium were used as pre-plant fumigants. The short interval between

fluensulfone application and planting could advantageous compared to using fumigant nematicides.

Yield increases were noted in every trial except for the spring of 2013 when there was little nematode population densities in the field. This phenomenon that is not uncommon where *Meloidogyne* spp. are concerned as yield reductions are only seen if harmful densities are present in the soil at time of planting (Schomaker and Been, 2013). Yield increases are attributed to nematode control since treatments that yielded best also had the lowest gall ratings.

These trials demonstrate that control of RKN may be obtained by an application of fluensulfone. However, results from these trials were inconsistent and further study to optimize application methods is warranted. Inconsistent results in controlling nematodes or increasing yield with non-fumigant nematicides are not unusual (Noling, 2005). When compared with fumigant nematicides, non-fumigant efficacy can appear erratic because non-fumigants are not volatile compounds and must rely on soil water to move throughout a treated area. In addition, non-fumigant nematicides control only nematodes and do not have the broad spectrum activity associated with many fumigants. Pulse irrigation contributed to nematode control in some trials but not in others so the benefit of utilizing that technique remains unclear. Small increases in horizontal water movement have been noted with use of pulse irrigation (Skaggs *et al.*, 2010) and it is reasonable to assume that a water soluble compound would distribute more efficiently through a raised bed using pulse irrigation. The registration of NIMITZ™ nematicide marks the first non-fumigant nematicide to receive a label for use in the U.S. in over 20 years and it is the only pesticide that is a fluoroalkenyl which separates it from the other non-fumigants

currently on the market. This unique chemistry, coupled with the expanding restrictions on soil fumigants (Noling *et al*., 2012), might help to insure that fluensulfone has a viable place in the market for nematode control in the foreseeable future. Additional research into the application methods, water volume usage during application, and plant-back intervals will be necessary to achieve optimal RKN control from fluensulfone.

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Tables:

Table 2.1 Amount of water applied (liters) during the pulse irrigation cycles.

Time	Spring 2012		Fall 2013		Spring 2013		Fall 2013	
	TRT C[x]	TRT D[y]	TRT C	TRT D	TRT C	TRT D	TRT C	TRT D
15 m ON	61.16	58.21	54.42	75.09	53.97	z	57.64	53.17
15 m OFF								
15 m ON	60.71	56.96	52.72	71.30	52.61		53.14	49.43
15 m OFF								
15 m ON	58.93	56.13	52.87	70.81	52.42		53.55	49.39
15 m OFF								
15 m ON	59.12	15.04	53.52	70.40	53.29		53.59	49.20
Total	239.93	215.44	213.54	287.62	212.30		217.94	201.21

[x]Fluensulfone application was made 1 hour before the start of pulse irrigation cycle.
[y]Fluensulfone application was made during the first 15 minutes of the first pulse irrigation cycle.
[z]Flow meter malfunctioned and data was not recorded.

Table 2.2 Pre-treatment population densities of juvenile *Meloidogyne incognita*[xy].

Treatment	Spring 2012	Fall 2012	Spring 2013	Fall 2013
Fluen. 3 kg a.i./ha PPI	272.8 a[z]	312.3 a	1.3 a	12.7 bc
Fluen. 3 kg a.i./ha NO Pulse	68.5 a	246.9 a	1.3 a	25.1 bc
Fluen. 3 kg a.i./ha WITH Pulse	57.8 a	363.7 a	0.7 a	40.9 abc
Fluen. 3 kg a.i./ha AT Pulse	43.8 a	322.9 a	0.7 a	56.1 ab
Oxamyl 22.5 kg a.i./ha PPI	193.5 a	454.9 a	0.7 a	10.5 c
Control	81.3 a	172.9 a	0.9 a	122.5 a

[x]Nematode counts have been transformed using $\log_{10}(x+1)$ to normalize data then a back transformation was made using 10^x.
[y]*M. incognita* J2s per 100 cm^3 of soil.
[z]Means are compared within columns. Means with the same letter do not significantly differ according Fisher's Protected LSD ($P \leq 0.05$).

Table 2.3 Post-treatment population densities of juvenile *Meloidogyne incognita*[xy].

Treatment	Spring 2012	Fall 2012	Spring 2013	Fall 2013
Fluen. 3 kg a.i./ha PPI	74.2 a[z]	39.2 a	2.2 a	5.0 a
Fluen. 3 kg a.i./ha NO Pulse	55.6 a	10.5 a	5.1 a	13.0 a
Fluen. 3 kg a.i./ha WITH Pulse	10.8 b	8.6 a	0.8 a	19.6 a
Fluen. 3 kg a.i./ha AT Pulse	7.4 b	36.4 a	3.7 a	10.0 a
Oxamyl 22.5 kg a.i./ha PPI	71.7 a	51.0 a	5.5 a	15.3 a
Control	76.7 a	18.8 a	8.4 a	7.5 a

[x]Nematode counts have been transformed using $\log_{10}(x+1)$ to normalize data then a back transformation was made using 10^x.
[y]*M. incognita* J2s per 100 cm^3 of soil.
[z]Means are compared within columns. Means with the same letter do not significantly differ according Fisher's Protected LSD ($P \leq 0.05$).

Table 2.4 Effect of nematicide treatment on plant vigor[x] during 4 experiments during 2012 and 2013.

2012

Treatment	Spring 14 DAP[y]	Spring 21 DAP	Spring 29 DAP	Fall 14 DAP	Fall 26 DAP
Fluen. 3 kg a.i./ha PPI	5.5 b[z]	5.3 b	5.2 b	5.5 a	4.8 a
Fluen. 3 kg a.i./ha NO Pulse	6.6 a	7.1 a	7.4 a	4.9 a	4.9 a
Fluen. 3 kg a.i./ha WITH Pulse	6.6 a	6.7 a	6.7 a	4.5 ab	4.4 a
Fluen. 3 kg a.i./ha AT Pulse	6.7 a	6.5 a	7.0 a	6.6 a	5.5 a
Oxamyl 22.5 kg a.i./ha PPI	6.1 a	5.6 b	5.3 b	4.9 a	3.6 a
Control	4.0 c	5.0 b	4.7 b	2.1 b	1.9 a

2013

Treatment	Spring 14 DAP[y]	Spring 21 DAP	Fall 14 DAP	Fall 19 DAP
Fluen. 3 kg a.i./ha PPI	7.5 a[z]	7.4 abc	6.6 a	7.3 a
Fluen. 3 kg a.i./ha NO Pulse	6.4 bc	6.8 c	5.5 bc	5.8 abc
Fluen. 3 kg a.i./ha WITH Pulse	5.9 c	6.8 c	5.3 c	4.6 cd
Fluen. 3 kg a.i./ha AT Pulse	6.4 bc	6.9 bc	5.0 c	4.8 bcd
Oxamyl 22.5 kg a.i./ha PPI	7.5 a	7.9 a	6.3 ab	6.4 ab
Control	6.0 ab	7.8 ab	4.6 c	3.5 d

[x]Vigor rated on a 0-10 scale with 0 being a dead plant and 10 being a vigorous healthy plant.
[y]DAP= Days after planting.
[z]Means are compared within columns. Means with the same letter do not significantly differ according Fisher's Protected LSD ($P \leq 0.05$).

Table 2.5 Number of cucumber plants per plot during the fall 2012 and fall 2013[y].

Treatment	Fall 2012	Fall 2013
Fluen. 3 kg a.i./ha PPI	11.3 ab[z]	14.8 a
Fluen. 3 kg a.i./ha NO Pulse	12.6 ab	13.4 a
Fluen. 3 kg a.i./ha WITH Pulse	11.8 ab	12.9 a
Fluen. 3 kg a.i./ha AT Pulse	13.7 a	12.5 a
Oxamyl 22.5 kg a.i./ha PPI	10.4 bc	11.8 a
Control	7.9 c	8.3 b

[y]Mean number of plants per plot 3 weeks after planting. Initially there were 15 plants per plot.
[z]Means are compared within columns. Means with the same letter do not significantly differ according Fisher's Protected LSD ($P \leq 0.05$).

Table 2.6 Cucumber root galling severity for the fall 2012 and spring 2013[y].

Treatment	Fall 2012	Spring 2013
Fluen. 3 kg a.i./ha PPI	7.2 a[z]	0.7 a
Fluen. 3 kg a.i./ha NO Pulse	4.8 a	0.4 a
Fluen. 3 kg a.i./ha WITH Pulse	5.8 a	0.4 a
Fluen. 3 kg a.i./ha AT Pulse	6.8 a	1.0 a
Oxamyl 22.5 kg a.i./ha PPI	6.6 a	1.2 a
Control	6.8 a	1.1 a

[y]Root gall ratings were conducted on a 0 to 10 scale with 0 being no visible galls on the roots and 10 being 100% of root system galled.
[z]Means are compared within columns. Means with the same letter do not significantly differ according Fisher's Protected LSD ($P \leq 0.10$).

Table 2.7 *Meloidogyne incognita* galling incidence on cucumber[y].

Treatment	Spring 2012	Fall 2012	Spring 2013	Fall 2013
Fluen. 3 kg a.i./ha PPI	100.0 a	100 a	76 ab	87 a
Fluen. 3 kg a.i./ha NO Pulse	86 b	100 a	59 bc	96 a
Fluen. 3 kg a.i./ha WITH Pulse	93 ab	100 a	50 c	98 a
Fluen. 3 kg a.i./ha AT Pulse	98 a	100 a	76 ab	85 a
Oxamyl 22.5 kg a.i./ha PPI	100 a	100 a	80 ab	100 a
Control	98 a	100 a	83 a	100 a

[y]Incidence is the percentage of exhumed roots that exhibited galling.
[z]Means are compared within columns. Means with the same letter do not significantly differ according Fisher's Protected LSD ($P \leq 0.10$).

Figures:

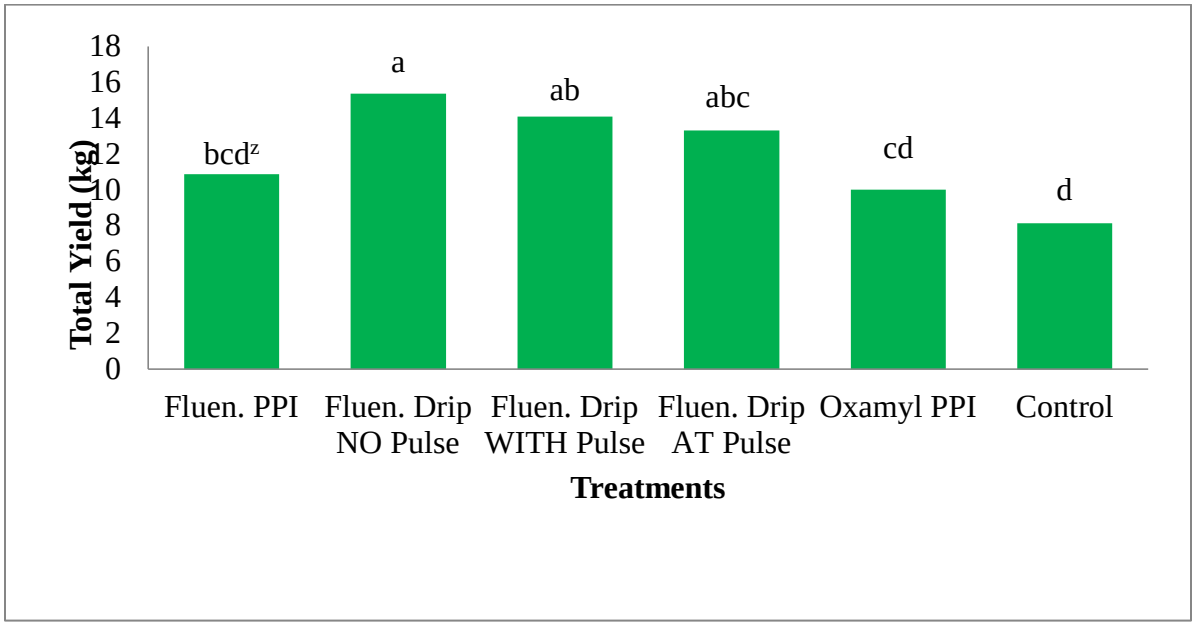

Fig 2.1a Total yield from 2012 spring field trial.
[z]Means with the same letter do not significantly differ according Fisher's Protected LSD ($P \leq 0.05$).

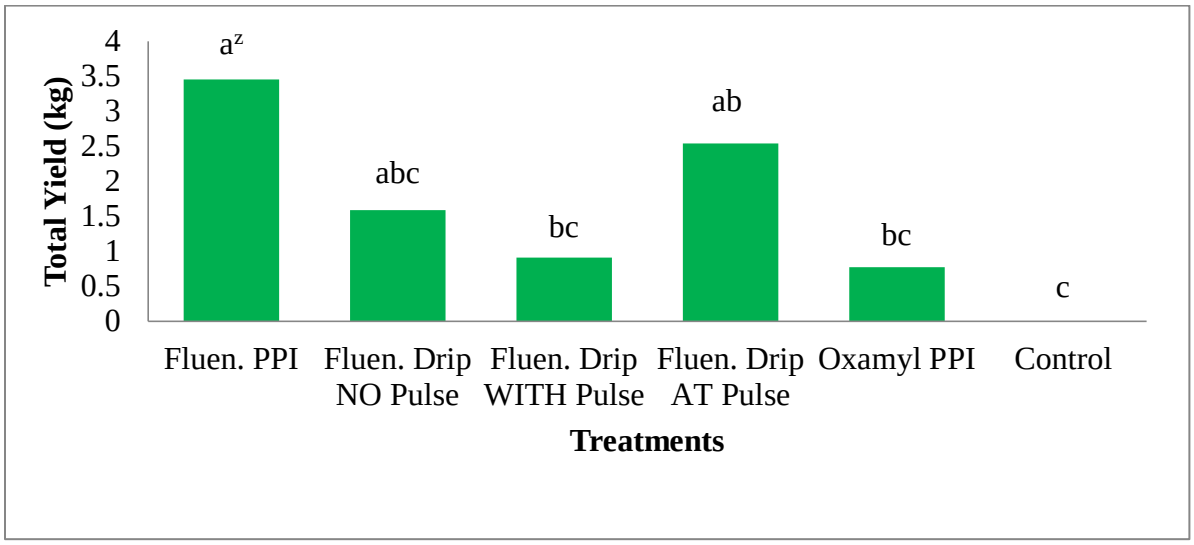

Fig 2.1b Total yield from 2012 field trial.
[z]Means with the same letter do not significantly differ according Fisher's Protected LSD ($P \leq 0.05$).

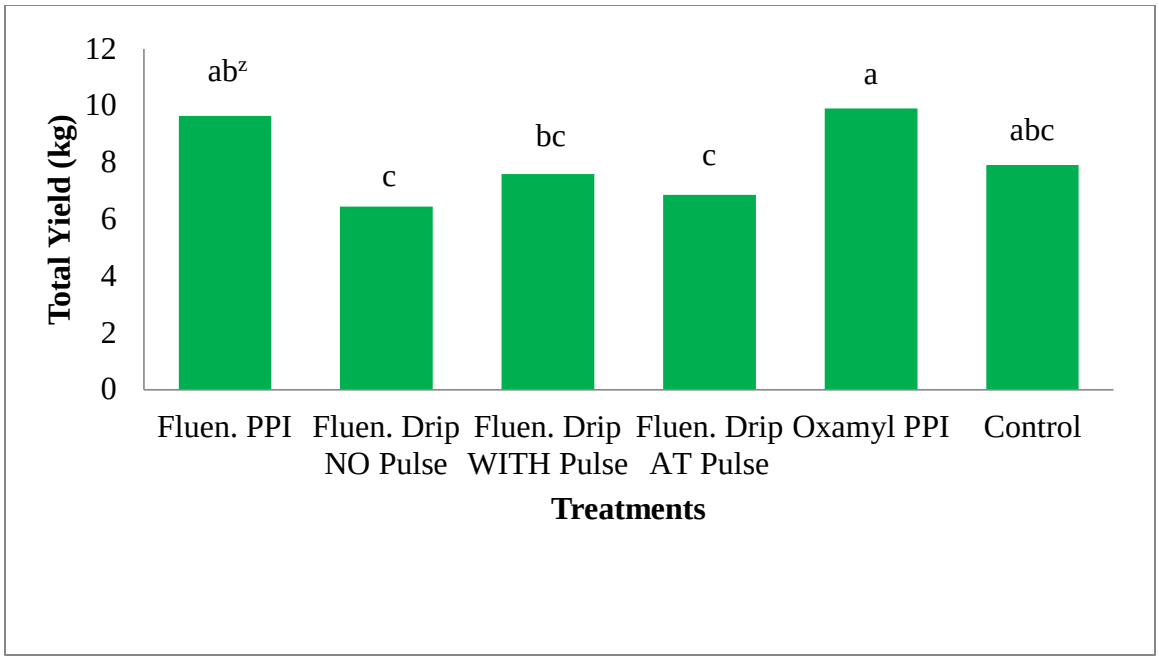

Fig 2.2a Total yield from 2013 spring field trial.
[z]Means with the same letter do not significantly differ according Fisher's Protected LSD ($P \leq 0.05$).

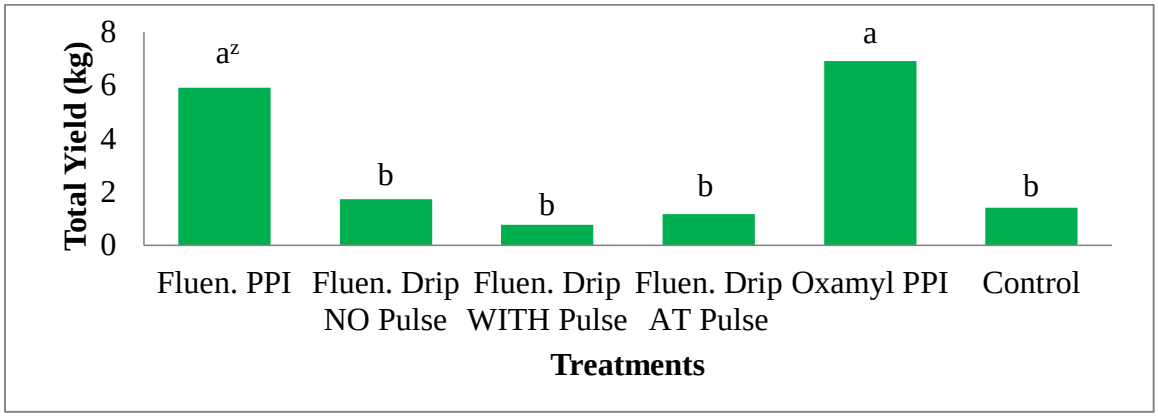

Fig 2.2b Total yield from 2013 fall field trial.
[z]Means with the same letter do not significantly differ according Fisher's Protected LSD ($P \leq 0.05$).

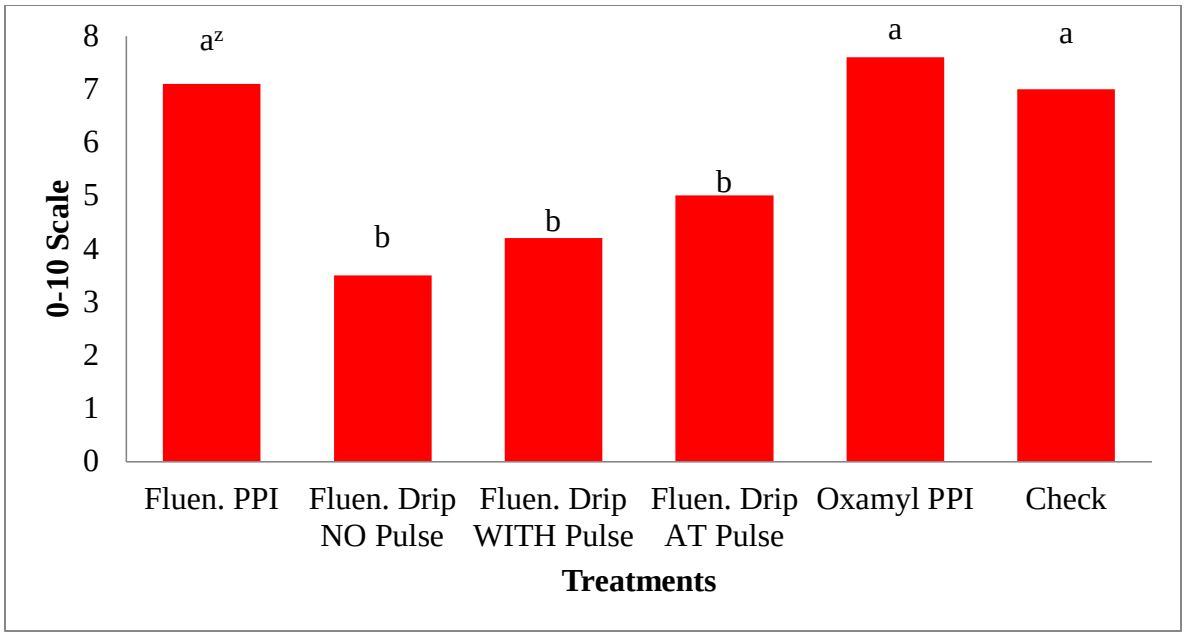

Fig 2.3a Spring 2012 gall ratings.
[z]Means with the same letter do not significantly differ according Fisher's Protected LSD ($P \leq 0.10$).

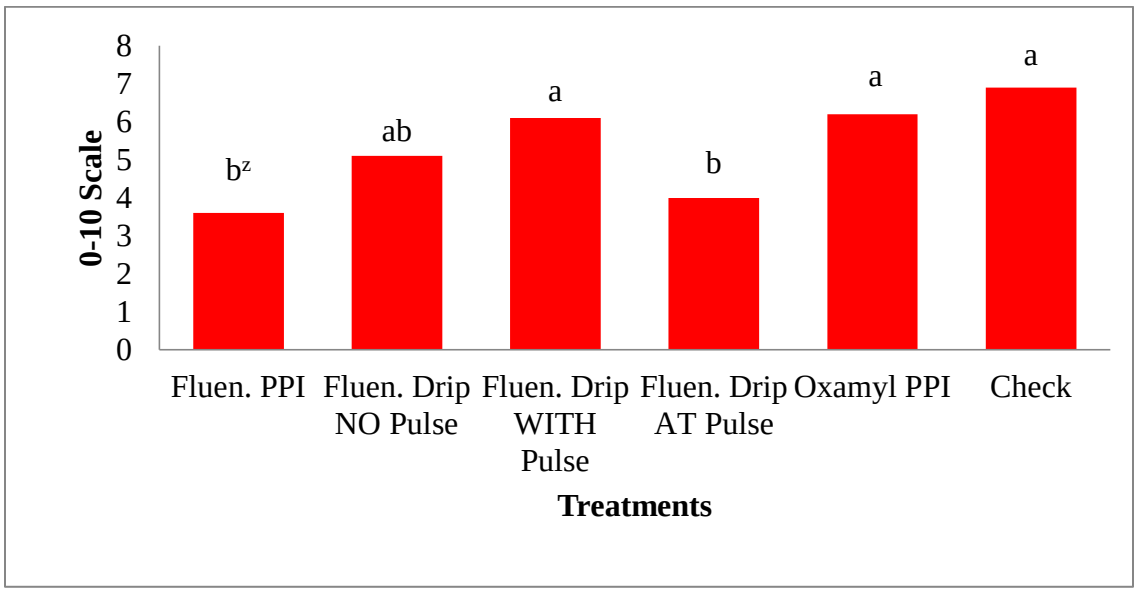

Fig 2.3b Fall 2013 gall ratings.
[z]Means with the same letter do not significantly differ according Fisher's Protected LSD ($P \leq 0.10$).

CHAPTER 3

EFFICACY OF FLUENSULFONE IN A TOMATO-CUCUMBER DOUBLE CROPPING SYSTEM

[1]Morris, K.A., D.B. Langston, D.W. Dickson, R.F. Davis, P. Timper, and J.P. Noe. To be submitted to *Pest Management Science.*

Abstract

Field trials were conducted in the spring and fall of 2013 and 2014 in Tifton, GA and Citra, FL to evaluate the efficacy of different control methods for nematodes in tomato-cucumber double cropping systems. The purpose of these trials was to determine what effect nematode control strategies applied to tomato in the spring have on nematode population levels and damage on a second crop of cucumber in the fall. Treatments in the spring were 1,3-dichloropropene (1,3-D) (112.0 L/ha), fluensulfone (3.0 kg a.i./ha), a resistant cultivar ('PS 01522935'), and an untreated control. All plots except for those planted to the resistant cultivar were planted with the cultivar 'Tribute'. The population density of J2s in the soil, plant vigor, yield, incidence, and galling severity ratings (0-10 scale) were recorded for spring and fall crops. There was no location x treatment interaction for tomato vigor, weight, galling or incidence data between locations so data was combined each year (P>0.05). There was no effect of nematicide treatment on vigor or yield of tomato. The 1,3-D, fluensulfone, and the resistant cultivar significantly decreased root galling by 91%, 73%, and 97%, respectively compared to an untreated control. The 1,3-D, fluensulfone, and resistant cultivar reduced galling incidence by 77%, 41%, and 89%, respectively compared to an untreated control. Tomato plots from the spring were divided into split-plots for the fall where the main plot was the treatment from the spring cucumber subplots were either treated with fluensulfone (3.0 kg a.i./ha. via drip irrigation) or left untreated. The fall application of fluensulfone significantly improved cucumber vigor and significantly reduced gall ratings compared to untreated subplots. The fall treatment increased cucumber yield in Citra, but not in Tifton. The

results suggest that fluensulfone can be used to manage root-knot nematode in a double-cropping system.

Introduction

Vegetable crops in the southeastern U.S. are commonly grown on plastic mulch. In addition to helping to retain moisture and fertilizer (Granberry, 2000), plastic mulch also increases soil temperature earlier in the season which allows for earlier planting dates compared to bare-ground systems (Lament, 1993). Soil fumigants are usually applied prior to plastic mulch application for control of soil-borne diseases, nematodes, and weeds. Once a fumigant has been applied, plastic mulch is laid over the treated area to help retain the fumigant in the soil which increases its efficacy and to increase application safety. Because of the expense associated with applying new plastic each time a crop is grown and removing the old plastic from the field, growers commonly grow multiple crops on one application of plastic mulch. This practice is known as double-cropping. A common double crop sequence in the Southeast U.S. is a tomato-cucurbit rotation. Tomatoes are planted on new plastic in the spring and then followed by a cucurbit crop in the fall. Several pests affect both tomato and cucurbit crops, including the root-knot nematode (RKN) or *Meloidogyne* spp. The galling symptoms caused by RKN infection lead to a yield reduction (Karssen *et al.*, 2013) and may predispose plants to secondary invaders (Back *et al*, 2002).

Controlling RKN on the second crop has been an area of interest with several studies evaluating different control options in double cropping systems. Desaeger and Csinos (2006) evaluated different fumigant nematicide programs for control of RKN on first crop tomato or eggplant followed by a second crop cucurbit. However, RKN also

can be managed with resistant crops. Root-knot nematode resistant tomato cultivars are available and some studies have documented the effect of these resistant cultivars on the damage incurred from RKN in the second crop (Theis *et al*., 2004; Hanna, 2000). The resistance to RKN in tomato is controlled by the *Mi* gene which has been bred into many tomato cultivars available today (Smith, 1944). Some studies have shown this resistance can be broken at soil temperatures >28 ^{o}C (Dropkin, 1969); however, Rich and Olson (1999) did not observe this in trials in northern Florida, but resistance has been broken by highly virulent populations of nematodes (Castagnone-Sereno, 1996; Kaloshian *et al*., 1996; Roberts *et al*., 1990).

Until recently, methyl bromide (MeBr) was the primary fumigant used on the first crop. MeBr has broad spectrum activity against a wide variety of pests, including nematodes. However, use of MeBr has been banned via the Montreal Protocol as it has been identified as an ozone depleting substance (U.S. Environmental Protection Agency, 2000). With the use of MeBr being phased-out, efforts have been made to discover alternatives for nematode management. Other fumigant nematicides are still available, including Telone® II (1, 3-dichloropropene), chloropicrin, and Paladin® (dimethyl disulfide). These fumigants are still the primary nematicide choice for first crop; although they are costly, difficult to apply, require buffer zones, have a long interval between treatment and planting (plant-back), and pose worker safety concerns. In addition, the future use of these products is unclear since they are heavily regulated by the EPA and may pose significant environmental safety concerns.

Fumigant nematicides applied to a first crop provide adequate nematode control; however, their efficacy may not persist long enough to provide satisfactory nematode

control on the second crop (Giannakou and Karpouzas, 2003; Lembright, 1990). The use of chisel injected fumigants on the second crop is not an option because the plastic mulch would be destroyed during application. Therefore, drip applied nematicides are used for RKN control on the second crop. Drip applied fumigants, such as Telone® EC (1,3-D) and Vapam® (metham sodium), may be applied through the drip irrigation system prior to planting the second crop. However, the soil distribution of drip applied fumigant nematicides can be limited since they have to move through the irrigation water before volatilizing (Csinos *et al*., 2002). The carbamate oxamyl is commonly applied as both a foliar and drip application for control of RKN on second crop plastic. However, oxamyl is not a nematicide, but rather a nematistat which temporarily paralyzes nematodes until concentrations of the active ingredient fall below a toxic level (Rich *et al.*, 2004; Wright, 1981); therefore, multiple applications of oxamyl must be made throughout the growing season to achieve adequate control.

Fluensulfone is a new non-fumigant fluoroalkenyl nematicide which received EPA registration in 2014 on cucurbit and fruiting vegetables crops under the trade name NIMITZ™. Fluensulfone may be applied with pre-plant incorporation or through drip irrigation. There is little published data on the efficacy of fluensulfone against RKN, but preliminary data suggests that fluensulfone can be an effective nematicide (Oka *et al.*, 2012; Csinos, *et al*., 2010). Our study aims to evaluate the efficacy of fluensulfone for control of RKN in a tomato-cucumber double-cropping system. Our specific objective is to evaluate the effect of fluensulfone on nematode population levels and damage to a fall cucumber crop following a spring tomato crop.

Materials and Methods

Site description and land preparation: Four field trials were conducted in the spring and fall of 2013 and 2014. Two trials were conducted each year at two separate locations: the University of Georgia Hort Hill Farm in Tifton, GA on a Tifton loamy sand (90% sand, 6% silt, 4% clay; pH 5.6; 1% organic matter; fine, loamy, kaolinitic thermic Plinthic Kandiudults) and the University of Florida Plant Science Research and Education Unit in Citra, FL on an Arredondo sand (97% sand, 2 % silt, 1 % clay; pH 6.4; 0.45% organic matter; loamy, siliceous, semiactive, hyperthermic, Grossarenic Paleudults). Trials consisted of a spring tomato crop followed by a fall cucumber crop. Tomato plots were arranged in a randomized complete block design with six replications. Cucumber plots utilized a split-plot design with six replications where the tomato plots from the spring were the main plot and cucumber plots were the sub-plots. Each location had a history of vegetable crops and a natural infestation of *M. incognita* in Tifton and *M. arenaria* and *M. javanica* in Citra.

In 2013, land was harrowed and roto-tilled on 25 February and 4 March in Citra and Tifton, respectively. In 2014, land was harrowed and roto-tilled on 12 March and 11 March for Citra and Tifton, respectively. Tomato plots were 10.7 m long and 0.8 m wide with a 2 m alley between plots.

General management: A single 10 mm drip line with 30.5 cm emitter spacing was placed 5 cm below the soil line at the same time as plastic was laid. Pre-plant fertilizer and subsequent drip fertilizer applications were made according to University of Georgia and University of Florida Extension recommendations. All herbicidal,

insecticidal, and fungicidal applications followed the University of Georgia and University of Florida Extension recommendations.

Treatments: First crop tomato treatments included Telone® II (1,3-D), fluensulfone pre-plant incorporation (PPI), a resistant cultivar ('PS 01522935', Seminis Seeds), and an untreated control. 1,3-dichloropropene (1,3-D) was applied as a pre-plant injection using a Yetter injection rig calibrated to deliver 112 L/ha on 5 March and 13 March in Citra in 2013 and 2014, respectively, and on 8 March and 14 March in Tifton for years 2013-14, respectively. Plastic was laid only to 1,3-D plots immediately after application using a tractor drawn bed-shaper and plots were covered with very impermeable film (VIF) plastic. Fluensulfone was applied to spring tomato crops as a PPI on 25 March and 9 April in Citra for years 2013 and 2014, respectively, and on 27 March and 10 April in Tifton for years 2013 and 2014, respectively. Treatments were applied using a CO_2 powered backpack sprayer with a four nozzle boom calibrated to deliver 187 L/ha. Fluensulfone was immediately incorporated into the soil using a PTO driven roto-tiller. Plastic mulch was then applied to the fluensulfone plots and to the resistant cultivar and untreated plots. Tomato seedlings ('PS 01522935' and 'Tribute') were transplanted on 9 April and 21 April in Citra in 2013-14, respectively, and on 8 April and 22 April in Tifton in 2013-14, respectively. Both tomato varieties are resistant to tomato yellow leaf curl virus and tomato spotted wilt virus.

The second crop cucumber plots were arranged in a split-plot design. Cucumber plots were 4 m long and 0.8 m wide with a 2 m alley between plots. Plots either received a fluensulfone drip application (3.0 kg a.i./ha) or were left untreated. Drip applications of fluensulfone were applied on 7 August and 4 August in Citra for years 2013 and 2014,

respectively, and 8 August and 5 August in Tifton for years 2013 and 2014, respectively. Beds were given a pre-treatment irrigation cycle of 1 hr to allow for adequate bed moisture at time of application. Fluensulfone was injected and then water was allowed to run for an additional 15 m to flush any remaining fluensulfone from the lines. Formulated fluensulfone was mixed in a 3 L bottle for injections in Tifton and was mixed in 48 L of water for injections in Citra. Untreated plots were given the same amount of water as treated plots. Thirteen cucumber seedlings, 'Impact', were transplanted into plots on 14 August and 18 August for Citra 2013 and 2014, respectively, and on 15 August and 19 August for Tifton 2013-14, respectively.

Data collection: In all trials, soil cores were collected from the middle of plots prior to treatment application and then again after root gall ratings to assess RKN population densities before and after treatment. Five soil cores were taken from the middle of each plot using 10.16 x 30.5 cm conical sampling probes. Samples were sent to the University of Georgia Nematology Lab (Athens, GA) and RKN juveniles were counted per 100 cm^3 of soil. Nematode count data was transformed using $\log_{10}(x+1)$ to normalize the data and then was back-transformed using 10^x to represent the number of J2s per plot.

Plant vigor was rated in all trials to evaluate treatment effects. Vigor ratings were evaluated on a 0 to10 scale with 0 being a dead plant and 10 being live, vigorous, healthy plant. Tomato was evaluated for vigor at 14 and 21 days after transplanting (DAP) in 2013 and 14, 21, and 28 DAP in 2014. A vigor rating was conducted on cucumber 14 and 28 DAP in 2013 and 21 and 28 DAP in 2014.

Each crop in each trial was harvested multiple times. In 2013, tomato plants were harvested on 13 June and 24 June in Citra and 17 June, 25 June, and 8 July in Tifton. In 2014, tomatoes were harvested on 24 June and 3 July in Citra and on 23 June and 2 July in Tifton. Every third plant in the plot was harvested for a total of eight plants per plot. All ripe fruit was picked for the first harvest and plants were stripped of all fruit for the final harvest. In 2013, cucumbers were harvested on 26 September, 30 September, 3 October, 7 October, and 10 October in Citra and 24 September, 1 October, and 8 October in Tifton. In 2014, cucumbers were harvested on 9 October and 15 October in Citra and 30 September, 9 October, and 13 October in Tifton.

Root were rated for galling at the end of each trial to evaluate RKN damage. Galling was rated on a 0 to 10 scale with 0 being no visible galls and 10 being 100% of the root system galled. In the tomato plots, a 2 m section in the middle of the plot was used to evaluate nematode galling. Five tomato plants from this section were exhumed and rated. This area then served as the alley between the split-plot cucumber plots. Gall ratings for tomato were conducted on 24 June and 8 July 2013 in Citra and Tifton, respectively, and on 3 July and 2 July 2014 in Citra and Tifton, respectively. Seven cucumber plants per plot were exhumed for gall rating on 10 October and 8 October 2013 in Citra and Tifton, respectively, and on 15 October and 13 October 2014 in Citra and Tifton, respectively. Galling incidence was measured by the percentage of exhumed plants that exhibited galling.

Statistical analysis: Data was analyzed using SAS (SAS Institute, Cary NC) GLIMMIX PROC to test for interactions between treatments and locations. Tomato data was analyzed as a randomized complete block design and cucumber data was analyzed as

a split-plot. All data was combined across years and, when possible, across locations when no treatment x location interaction existed ($P>0.05$). Year was considered a random variable. All mean differences are reported according to Student's *t*-tests $\alpha=0.05$ using the PDIFF operation.

Results

Main treatment in tomato: There was no significant treatment x location interactions for vigor, weight, galling, or incidence so data was combined between locations (Table 3.1). Main treatments did not have a significant effect on plant vigor ($P=0.6537$) at 14 d after treatment compared to an untreated control. Vigor data was similar for other rating dates (data not shown). Likewise, the treatments did not have an effect on plant yield ($P=0.1728$). Gall ratings were significantly lower among treated plots compared to untreated controls ($P<0.0001$). The resistant cultivar had the lowest gall ratings but was not significantly lower than the 1,3-D treatment. Fluensulfone had more galling than the resistant cultivar, but there was no difference between fluensulfone and 1,3-D. Galling incidence was lower among treated plots compared to an untreated control with the resistant cultivar and 1,3-D having the lowest galling incidence ($P<0.0001$). There was a significant treatment x location interaction for nematode numbers ($P=0.0004$) (Table 3.2). In both locations, all nematicide treatments significantly reduced the number of J2s in the soil at harvest, but there were no differences among the nematicide treatments.

Sub-plot treatments in cucumber: There were no significant treatment x location interactions for vigor, galling, or nematode population densities so this data was combined between locations (Table 3.3). Both the main-plot ($P=0.0066$) and sub-plot

(P=0.0060) treatment significantly affected cucumber vigor. The application of fluensulfone to second crop cucumber significantly improved plant vigor 14 days after treatment when compared to untreated plots when means were averaged across main-plot treatments. The untreated spring plot followed by an untreated fall plot had the lowest numerical vigor ratings. The 1,3-D treatment had increased vigor compared to an untreated check regardless of whether a sub-plot treatment of fluensulfone was applied or not. Vigor was improved over the untreated when fluensulfone or the resistant cultivar was the main-plot treatment only if fluensulfone was applied again to the second crop.

Cucumber gall ratings were reduced by both the main-plot (P<0.0001) and sub-plot (P=0.0023) treatments. Cucumber gall ratings were significantly less when fluensulfone was the main-plot treatment versus an untreated sub-plot when means were averaged across main-plot treatments. The 1,3-D and resistant cultivar main-plot treatment had significantly lower gall ratings compared to the untreated check. The fluensulfone main-plot treatment significantly reduced root galling compared to an untreated when a sub-plot treatment of fluensulfone was applied, but not when sub-plots were untreated. Neither the main-plot treatment (P=0.2296) or the sub-plot treatment (P=0.0772), had a significant effect on the number of J2s in the soil at harvest.

There was a significant treatment x location interaction for the sub-plot treatment effect on cucumber weight and galling incidence (Table 3.4). The main-plot treatment (P<0.0001), but not the sub-plot treatment (P=0.2852), had an effect on cucumber yield per plot in Tifton, while both the main-plot and sub-plot treatment had a significant effect on yield in Citra (P<0.0001 and P=0.0027, respectively). The sub-plot treatment of fluensulfone significantly increased cucumber yield in Citra compared to untreated sub-

plots when yields were combined across main-plot treatments. Galling incidence was affected by the main-plot treatment (P=0.001), but not the sub-plot treatment (P=0.8461) in Tifton. The opposite effect happened in Citra, where the main treatment did not affect galling incidence (P=0.0882), but the sub-plot treatment did (P=0.0004): a fluensulfone application to the second crop significantly reduced galling incidence when compared to an untreated sub-plot treatment when means were averaged across whole-plot treatments.

Discussion

There was no main-plot x sub-plot interaction for any variable tested; therefore, the effect of a fall application of fluensulfone is not influenced by the spring treatment. Obtaining satisfactory efficacy through the use of a drip-applied non-fumigant nematicide can be difficult to achieve (Noling, 2005). Colyer *et al*. (1998) demonstrated that the use of a resistant cultivar as a spring treatment was better than the non-fumigant ethoprop in controlling RKN in a tomato-cucumber double cropping system. Our results suggest that a spring application of fluensulfone is as effective as a resistant cultivar in protecting a second crop and can be a beneficial tool in double-cropping systems. Although our study evaluated a different nematicide, ethoprop and fluensulfone are both non-fumigants. However, their respective modes of actions are different. Oka *et al.* (2009) determined that fluensulfone was a true nematicide, with further studies proving that its mode of action is different from the organophosphates and carbamates (Kearn *et al.*, 2014). That fluensulfone kills nematodes rather than paralyzing them is a likely reason that fluensulfone reduced nematode galling on cucumber.

Oxamyl, a carbamate, is used with pre-plant incorporation, drip, and foliar application for control of RKN on vegetable crops and reduces galling and improves

yield (Gugino *et al*., 2006; Giannakou *et al*., 2005; Giannakou *et al*., 2003). However, multiple applications throughout the growing season are needed to achieve satisfactory control. In contrast, satisfactory nematode control was obtained in these studies with a single drip application of fluensulfone. However, the possibility of using fluensulfone as a pre-plant nematicide followed by in-season applications of oxamyl has not been evaluated and future studies should examine the possibility of using fluensulfone in tandem with other non-fumigants.

There was a significant treatment x location interaction for galling incidence and yield of cucumber. The Arredondo sand in Citra has less clay content and less organic matter than the Tifton loamy sand in Tifton. Fluensulfone has recently been shown to have an affinity to bind to clay and organic matter content in the soil (Oka *et al*., 2013). Possibility that some of the fluensulfone was bound to soil particles in Tifton may help explain why the sub-plot treatment had a significant effect on yield and galling incidence in Citra but not in Tifton. However, Morris *et al.*(2015 unpublished) found that fluensulfone had an affinity to adsorb to an Arredondo sand which could contradicts the hypothesis that adsorption of the pesticide played a role in control. Additional research should be conducted on soils with varying textures and characteristics to evaluate the efficacy of fluensulfone on different soil types. An increase in the volume of water used to apply fluensulfone in Citra could have helped to distribute the product throughout the bed, resulting in increased efficacy versus Tifton.

Nordmeyer and Dickson (1989) found *M. javanica* to be more sensitive to treatments of aldicarb than *M. arenaria* or *M. incognita.* Since the populations in Citra contain high densities of *M. javanica* it could be that this species is more susceptible than

the high populations of *M. incognita* in Tifton. Thus, leading to increased yield and decreased galling in Citra.

Although the primary objective our study was to evaluate the efficacy of fluensulfone when applied through a drip system to a second crop, the efficacy obtained by the pre-plant incorporation application of fluensulfone on first crop tomato is important. Non-fumigant organophosphates and carbamates are often less effective than the fumigant 1,3-D (Giannakou *et al.*, 2002). However, in our study, fluensulfone provided the same reduction of RKN galling on tomato as a 1,3-D application. Fluensulfone also reduced the number of J2s in the soil after tomato harvest to the same level as a resistant cultivar or the 1,3-D application. The pre-plant incorporation is a broadcast spray that is then mechanically incorporated into the soil. It does not have to rely on irrigation water to move it to the target zone which is a distinct disadvantage to using drip applied pesticides (Desaeger and Csinos, 2006).

In conclusion, our studies demonstrate that fluensulfone is an effective tool for managing RKN in double cropping systems. In addition, the lower worker safety concerns, ease of application, no post-application re-entry period, and no requirement for buffer zones associated with using fluensulfone make it a desirable alternative to the more hazardous fumigants.

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Tables

Table 3.1 Effect of nematicide treatment on vigor, weight, root-knot galling, and incidence of galled tomato roots in Citra FL and Tifton, GA in 2013 and 2014.

Treatment	Vigor[v]	Weight[w]	Galling[x]	Incidence[y]
Telone II	6.71 a[z]	19.05 a	0.35 bc	21.45 c
Fluensulfone	7.41 a	22.03 a	1.10 b	53.75 b
Resistant Cultivar	6.67 a	17.77 a	0.11 c	9.92 c
Untreated	7.16 a	19.60 a	4.11 a	91.71 a
	P-value	*P*-value	*P*-value	*P*-value
Treatment	0.6537	0.1728	<0.0001	<0.0001
Location*Treatment	0.7833	0.4467	0.1882	0.7608

[v]Plant vigor 14 days after planting. Vigor was recorded on a 0 to 10 scale with 0 being a dead plant and 10 being a completely healthy plant.
[w]Total fruit weight (kilograms).
[x]Gall ratings were conducted on a 0 to 10 scale with 0 being no visible galls and 10 being 100% of root system galled.
[y]The percentage of examined roots that were galled.
[z]Means are compared within columns. Means with same letter are not significantly different according to t-test α=0.05.

Table 3.2 Effect of nematicide treatment on the population densities of *Meloidogyne incognita* in soil after tomato harvest.

	Tifton, GA[x]	Citra, FL
Treatment	RKN Density[y]	RKN Density
Telone II	18.75 b[z]	13.92 b
Fluensulfone	274.42 b	48.66 b
Resistant Cultivar	24.91 b	12.08 b
Untreated	869.67 a	186.92 a
	P-value	*P*-value
Treatment	<0.0001	0.0005

[x] Nematode counts have been transformed using $_{log}(x+1)$ to normalize data and then a back transformation was made using 10^x.
[y] Data was collected over two field trials in 2013 and 2014. Data is not combined among locations because of a significant treatment x location interaction (P=0.0004). RKNs were counted per 100 cm^3 of soil recorded from each plot.
[z] Means are compared within columns. Means with same letter are not significantly different according to t-test α=0.05

Table 3.3 Effect of sub-plot treatment on the vigor, galling, and root-knot nematode counts on cucumber.

Treatment	Vigor[u]	Galling[v]	RKN Counts [wx]
Telone II-Fluen. Drip	5.46 a[y]	2.73 c	2.79 b
Telone II-Untreated	5.04 a	3.54 bc	8.59 ab
Fluen. PPI-Fluen. Drip	4.88 a	3.09 c	7.75 ab
Fluen. PPI-Untreated	4.46 ab	5.19 ab	22.72 a
Resistant-Fluen. Drip	4.99 a	2.94 c	8.56 ab
Resistant-Untreated	4.54 ab	3.56 bc	15.29 ab
Untreated-Fluen. Drip	4.69 ab	4.85 ab	15.76 ab
Untreated-Untreated	3.67 b	6.01 a	20.47 ab
Fluensulfone	5.02 A[z]	3.40 B	9.12 A
Untreated	4.43 B	4.58 A	17.22 A
	P-value	*P*-value	*P*-value
Main-plot Treatment	0.0066	<0.0001	0.2296
Sub-plot Treatment	0.0060	0.0023	0.0772
Main-plot*Sub-plot	0.7251	0.5809	0.8855
Location*Sub-plot Treatment	0.1622	0.4185	0.5505

[u]Plant vigor 14 days after planting. Vigor was recorded on a 0 to 10 scale with 0 being a dead plant and 10 being a healthy plant.
[v]Gall ratings were conducted on a 0 to 10 scale with 0 being no visible galls and 10 being 100% of root system galled.
[w]RKNs were counted per 100 cm^3 of soil recorded from each plot.
[x]Nematode counts have been transformed using $_{log}(x+1)$ to normalize data and then a back transformation was made using 10^x.
[y]Means are compared within columns. Means with same lowercase letter are not significantly different according to t-test α=0.05.
[z]Means are compared within columns. Means with same uppercase letter are not significantly different according to t-test α=0.05.

Table 3.4 Effect of sub-plot treatment on the fruit weight and incidence of nematode galling on cucumber.

	Tifton, GA[u]	Citra, FL	Tifton, GA[v]	Citra, FL
Treatment	Weight[w]	Weight	Incidence[x]	Incidence
Telone II-Fluen. Drip	2.69 a[y]	6.35 a	67.2 b	71.42 b
Telone II-Untreated	2.20 ab	2.66 b	60.8 b	83.33 ab
Fluen. PPI-Fluen. Drip	1.39 bcd	3.94 ab	72.7 b	85.03 ab
Fluen. PPI-Untreated	0.83 dc	2.50 b	76.3 ab	95.25 a
Resistant-Fluen. Drip	2.26 ab	3.73 ab	77.6 ab	73.83 b
Resistant-Untreated	2.02 abc	1.91 b	73.2 b	94.75 a
Untreated-Fluen. Drip	0.49 d	2.99 b	97.3 a	82.16 ab
Untreated-Untreated	0.31 d	1.49 b	95.4 a	98.83 a
Fluensulfone	1.72 A[z]	4.35 A	79.59 A	78.13 B
Untreated	1.36 A	2.14 B	78.47 A	93.04 A
	P-value	*P*-value	*P*-value	*P*-value
Main Treatment *P*=	<0.0001	<0.0001	0.0010	0.0882
Sub Treatment *P*=	0.2852	0.0027	0.8461	0.0004
Main*Sub *P*=	0.9903	0.5492	0.9035	0.7195

[u]Data was collected over two field trials in 2013-14. Data is not combined among locations because of a significant interaction (*P*=0.0131).
[v]Data was collected over two field trials in 2013-14. Data is not combined among locations because of a significant interaction (*P*=0.0347).
[w]Total fruit weight (kilograms).
[x]The percentage of examined roots that were galled
[y]Means are compared within columns. Means with same lowercase letter are not significantly different according to t-test α=0.05.
[z]Means are compared within columns. Means with same uppercase letter are not significantly different according to t-test α=0.05.

CHAPTER 4

SYSTEMIC ACTIVITY OF FLUENSULFONE ON FOUR VEGETABLE CROPS

[1]Morris, K.A., D.B. Langston, J.P. Noe, R.F. Davis, P. Timper, and D.W. Dickson. To be submitted to *Pest Management Science.*

Abstract

Fluensulfone is a new nematicide that is a member of the flouroalkenyl chemical group. The activity and phytotoxicity of this compound was tested during the spring of 2012 on four different vegetable crops: tomato ('Florida 47'), eggplant ('Night Shadow'), cucumber ('Rockingham'), and squash ('Payroll'). Seedlings of each crop were planted into 7 cm x 25 cm black cone-tainers containing a potting mix of 3:3:1 sand, pasteurized field soil, and a germinating mix, respectively, and incubated in a growth chamber at 28^{o}C, 75% humidity, with a 12 hr photoperiod. Cone-tainers were arranged in a randomized complete block design with six replications per treatment. Nematicide treatments were applied two days after transplanting and cone-tainers were inoculated with 1,500 *Meloidogyne incognita* J2s two days after nematicide treatment. Treatments consisted of fluensulfone at rates of 3, 6, and 12 g a.i/L, oxamyl at a rate of 4.8 g a.i./L, an inoculated nontreated control, and a non-inoculated nontreated control. Treatments were applied via backpack sprayer calibrated to deliver 234 L/ha. Prior to being treated, the base of the plant was secured tightly and the potting mix covered with plastic wrap and rubber bands to prevent nematicide contact with the soil. Plant heights and vigor was recorded 12 d after treatment to assess phytotoxicity. Four weeks after inoculation, dry weights of plant foliage, the number of root galls, and stained nematodes were determined. Tomato was the only crop species tested in which basipetal systemic activity of either nematicide was observed. In trials 1 and 2, the 6 g a.i./L and the 12 g a.i./L rate of fluensulfone reduced nematode numbers in tomato roots by 35-37% and 39-58%, respectively, compared to the nontreated control. The 12 g a.i./L rate of fluensulfone reduced nematode galling by 24-47%. The 6 g a.i./L rate of fluensulfone reduced

nematode galling by 32% in trial 2 but did not reduce galling in trial 1. Oxamyl significantly reduced nematode numbers in roots and galling on tomato by 58-74% and 51-73%, respectively, compared to the nontreated control. In trial 1, the high rate of fluensulfone reduced plant vigor and height in eggplant and cucumber. Additionally, reduced vigor of tomato was observed for all rates of fluensulfone. Phytotoxicity was not observed in squash. In trial 2, eggplant was the only crop to exhibit a reduction in vigor from fluensulfone. Phytotoxicity was not observed in any crop or trial for the oxamyl treatment. Fluensulfone has basipetal systemic activity when applied as a foliar spray in certain crops, such as tomato, but also can be phytotoxic to other crops, such as eggplant. Oxamyl only showed basipetal systemic activity in tomato in these experiments. Similar to fluensulfone, the basipetal systemic activity of oxamyl appears to be crop-dependent.

Introduction

Plant-parasitic nematodes are responsible for an estimated 14% of all crop losses worldwide (Mitkowski and Abawi, 2003). Of the 197 genera of plant-parasitic nematode described, the root-knot nematode , *Meloidogyne* spp, is one of the most devastating to vegetable crops (Handoo, 1998). Root-knot nematodes (RKN) are endoparasitic nematodes which, upon infection, create large galls within plant roots that inhibit the uptake of water and nutrients leading to a reduction in yield (Karssen *et al.*, 2013). In developed countries, plant-parasitic nematodes have historically been controlled through the use of fumigant nematicides. There have been a number of Environmental Protection Agency (EPA) registered fumigants on the market since Christie and Cobb (1940) first reported on the efficacy of methyl bromide (MeBr). However, due to environmental concerns, many of these fumigant nematicides, including MeBr, have been banned by the

EPA (Lambert and Bekal, 2002). The loss of MeBr has generated an increased interest in the use of non-fumigant nematicides.

The most widely used non-fumigant nematicides are the carbamates and organophosphates (Rich *et al.*, 2004). Both of these chemistry classes are acetyl cholinesterase inhibitors which do not kill nematodes but paralyze them for the period of time in which the active ingredient is above a toxic level (Opperman and Chang, 1990). Carbamates and organophosphates are generally applied to soil; however, some have been shown to have systemic activity within plants. Ease of application and the reduction in the potential for groundwater contamination are concerns that can be alleviated when foliar systemic nematicides are used versus a soil applied nematicides. Fenamiphos, an organophosphate, has been shown to have systemic activity against *M. hapla* when applied to the leaves of redcurrant, *Ribes rubrum* (Santo and Bolander, 1979). The systemic activity of oxamyl, a carbamate, is well documented (Lawrence and McLean, 2000; Wright and Womac, 1981; Potter and Marks, 1976; Rich and Bird, 1973). Oxamyl is commonly applied to the foliage of plants for control of parasitic nematodes and is known to have ambimobile translocation within plants (Hsu and Kleier, 1996; Peterson *et al.*, 1978).

Fluensulfone is a new non-fumigant nematicide in the fluoroalkenyl chemical class which received an EPA registration in September 2014 for control of plant-parasitic nematodes in cucurbits and fruiting vegetables. It has an unknown mode of action (Kearn *et al.*, 2014) but is a true nematicide (Oka *et al*., 2009). Reports on appropriate application methods and the efficacy of fluensulfone are limited but show significant efficacy of fluensulfone in reducing damage from RKNs (Csinos *et al.*, 2010; Karmon *et*

al., 2010). The systemic activity of fluensulfone is not well defined on a broad range of crops. Oka *et al.* (2012) reported that a foliar application of fluensulfone on pepper can control *M. incognita*. The objective of this study was to investigate the basipetal systemic activity of fluensulfone on different vegetable crops.

Materials and Methods

Nematode inoculum: Eggplant ('Black Beauty') roots were inoculated with *M. incognita* and allowed to grow for 65 d. Infested roots were excised, placed on hardware cloth over a large pan, and placed in a mist chamber for 7 d. Juvenile (J2) nematodes were collected daily by passing the water that accumulated in the pans through a 200 mesh sieve over a 500 mesh sieve. J2s were washed from the 500 ml sieve into a 500 ml flask and enumerated using a dissecting microscope (40 x). J2s were stored in a refrigerator at 4.4 ^{o}C for 7 d until time of inoculation.

Plant material: Two-week-old tomato ('Florida 47'), eggplant ('Night Shadow'), cucumber ('Rockingham'), and squash ('Payroll') were purchased from Lewis Taylor Farms, Tifton, GA and used in all trials. All transplants had at least two true leaves at time of planting. Crops were planted on 20 February and 25 June for trial 1 and 2, respectively. Seedlings were transplanted into black cone-tainer pots (5 cm x 25 cm) that were filled with 3:3:1 sand, pasteurized field soil, and Fafard's® Germinating mix. Plants were then placed in a growth chamber at 28^{o}C, 75% humidity, with 12 hr photoperiod for 2 d before treatment was applied.

Growth chamber trials: This experiment was conducted in the spring and summer of 2012 on University of Georgia campus, Athens, GA in Department of Plant

Pathology growth chambers. Treatments consisted of a foliar application of fluensulfone (ADAMA Agricultural Solutions Ltd. Raleigh, NC) at a rate of 3, 6, and 12 g a.i/L, oxamyl (DuPont Crop Protection Wilmington, DE) at a rate of 4.8 g a.i/L, an untreated inoculated control (positive control), and an untreated non-inoculated control (negative control). Both positive and negative controls received a foliar application of water at the same time nematicide treatments are applied. Foliar sprays were applied using a backpack sprayer calibrated to deliver 234 L/ha using 8004 T-Jet® tips. The soil surface was covered with Glad® ClingWrap which was sealed to the pot with a rubber band (Fig. 4.1) to prevent nematicide contact with the soil and ensure that the nematicide only contacted the plant foliage. The cover was removed after treatments had dried. After treatment, plants were placed back into the growth chamber for an additional 2 d before being inoculated with nematodes. Plants were inoculated with *M. incognita* juveniles by pipetting 1,500 juvenile nematodes into three holes 2.5 cm from the base of the transplants. Plants were returned to the growth chamber for a period of 4 weeks and were watered daily using an overhead wand nozzle and fertilized once per week with Miller® NutriLeaf 20-20-20 water soluble fertilizer. Each treatment had six replications and were arranged in a randomized complete block design. The experiment was conducted twice.

Data collection and statistical analysis: Plant heights were recorded and a vigor rating was made12 d post-treatment for evaluation of phytotoxicity. At 28 d after treatment, plant tops were cut from the soil line, placed in paper bags, and placed in a drying over at 60 ^{o}C for 48 hr and then weighed. Nematicidal activity of treatments was evaluated by washing the soil from roots and examining a 1.5 g representative root sample that was stained via the NaOCl-acid-fuchsin-glycerin technique (Byrd *et al*.,

1983) 28 d after treatment. Female root-knot nematodes and nematode galls within the sample were counted. The number of females and galls found in a sample was used to estimate the total number of nematodes and galls on the root system. All data was subjected to analysis of variance using Fischer's test of LSD to separate the means ($P<0.10$). All data analysis was conducted using ARM statistical software (Gylling Data Management, Brookings, SD).

Results

Effect of nematicide treatment on plant vigor: All rates of fluensulfone significantly reduced plant vigor of eggplant for trial 1 and 2, and of tomato for trial 1 compared to the positive control (Table 4.1). A significant reduction in the vigor of cucumber was observed in trial, but not trial 2, when fluensulfone was applied at a rate of 6 g a.i./L and 12 g a.i./L. There was no reduction in plant vigor for squash during either trial.

Effect of nematicide treatment on plant height: A significant reduction in plant height was recorded for eggplant during trial 1 in treatments receiving fluensulfone at a rate of 12 g a.i./L (Table 4.2). A significant reduction in plant height was recorded on tomato during trial 1 at the 3 g a.i./L rate of fluensulfone, but not at higher rates. Fluensulfone did not affect plant height for eggplant or tomato during trial 2. There were no significant reductions in plant height for cucumber or squash in either trial.

Effect of nematicide treatment on plant dry weight: A significant reduction of plant dry weight was recorded for eggplant (Table 4.4) in trial 1, but not trial 2, with a rate of 12 g a.i./L fluensulfone. A significant increase in dry weight occurred in tomato when fluensulfone was applied at a rate of 6 g a.i./L compared to both the lower and

higher rate of fluensulfone and the inoculated untreated control. However, there was no difference in dry weight observed in trial 2 on tomato. All inoculated cucumber treatments had significantly lower dry weights compared to the non-inoculated untreated control in trial 1. There are no differences in dry weight of cucumber during trial 2 or of squash in trial 1 or 2.

Effect of nematicide on nematodes and galling: Tomato was the only crop tested in which there as a significant reduction in the number of nematodes (Fig. 4.2) or galls (Fig. 4.3) when fluensulfone or oxamyl was applied. In trial 1, the 6 and 12 g a.i./L rate of fluensulfone reduced the number of female *M. incognita* in the root but only the 12 g a.i./L rate reduced galling compared to the positive control. In trial 2, the 6 and 12 kg a.i./L rate of fluensulfone reduced both the number of galls and the number of female *M. incognita* compared to the inoculated untreated control. Oxamyl reduced both galling and nematode infection in both tomato trials. There was no significant reduction in the number of nematodes (Table 4.4) or galls (Table 4.5) found in the roots of eggplant, cucumber, or squash for any treatment in either trial.

Discussion

In these studies fluensulfone and oxamyl showed basipetal systemic activity only on tomato. These findings agree with previous systemic studies using oxamyl on tomato (Stephan and Trudgill, 1983), but are the first report of the systemic activity of fluensulfone on tomato. Whether a pesticide will move symplasticly in plants is dependent upon a variety of factors including the characteristics of the leaf surface, physiochemical properties of the active ingredient, and whether or not an adjuvant was used (Wang and Liu, 2007).

Our results contradict previous studies on the systemic mobility of oxamyl for control of *M. incognita* on cucumber (Wright *et al*. 1980). There are no definitive reports on systemic mobility of oxamyl for control of root-knot nematodes in eggplant and squash. It is largely assumed, however, that systemic mobility would occur and oxamyl is labelled for foliar application for control of root-knot nematodes in eggplant and squash. However, the results in this study do not support the label recommendations. The label recommends that multiple foliar applications be made beginning 2 weeks after planting with a second application 2 weeks later for suppression of root-knot nematodes. It is possible that the single application of oxamyl used in this study was not sufficient to reduce nematode damage.

For a pesticide to be systemically moved in a plant, it must first penetrate the plant cuticle. Systemic activity of fluensulfone and oxamyl was not observed in squash, cucumber, or eggplant. The permeability of the plant cuticle in these crops could be abating the absorption of these pesticides into the plant leaf and there by prohibiting mobility of the pesticide.

The physiochemical characteristics of a pesticide can influence its absorption into the leaf as well. The rate at which a compound is diffused across a plant membrane is negatively correlated to the molecular weight of the compound (Bauer and Schönherr, 1992). The smaller the compound the greater the probability that it will be absorbed into the plant. The molecular weight of oxamyl is 219 D while the molecular weight of fluensulfone is 291.5 D. The molecular weight of fluensulfone and oxamyl are not likely to be a limiting factor on whether or not each respective compound could be absorbed

into a leaf since both of these pesticides have a molecular weight smaller than the exclusion limit (1000 D) of a plants plasmodesmata (Oparka and Roberts, 2001).

Plant vigor was reduced in cucumber, tomato, and eggplant in one trial, but only eggplant vigor was reduced in the second trial. It is possible that the environment contributed to the phytotoxicity observed in trial 1, but not trial 2. In trial 1 treatments were applied on a cloudy day with a daytime high of 18.8 ^{o}C. The treatments in trial 2 were applied on a clear sunny day with a daytime high of 29.8 ^{o}C. Although there are no published reports of the photodegradation of fluensulfone or of any other fluoroalkenyl class pesticides, the sunlight in trial 2 could have increased photodegradation of fluensulfone thereby reducing the phytotoxicity that was observed. The photodegradation of some pesticides, such as certain carbamates, can be quite rapid with studies showing that <20 % of the intial concentration applied remaining 12 h after application (Samanidou and Fytianos, 1988). More research is needed to evaluate the possibility of photodegradation of fluensulfone.

The fluensulfone used in these trials was formulated as an emulsifiable concentrate (EC) while the oxamyl treatment was formulated as a water soluble liquid. Phytotoxicity was observed only with the fluensulfone treatments. It is unclear, however, if fluensulfone was the phytotoxic compound or if it was the EC formulation which caused the crop injury. Emulsifiable concentrate formulations contain solvents and emulsifiers that have been reported to damage plant foliage (Hata and Hara, 1988). Oxamyl, a carbamate, has been known to provide enhanced growth and vigor in plants (Rethwisch and Kruse, 1998).

Although fluensulfone has shown promise when applied to soil prior to planting (Csinos *et al.*, 2010; Karmon *et al.*, 2010), this study shows that the lack of basipetal systemic mobility within cucumber, squash, and eggplant, coupled with the possibility of crop injury concerns, makes a foliar application of fluensulfone not feasible. However, since basipetal systemic activity for control of *M. incognita* has been observed with pepper (Oka, 2012) and now tomato, the potential for foliar application will need to be evaluated on a case by case basis for different crop species.

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Tables

Table 4.1 Effect of foliar applied nematicide on plant vigor[y] over 2 trials

Treatment	Squash 1	Squash 2	Cucumber 1	Cucumber 2	Eggplant 1	Eggplant 2	Tomato 1	Tomato 2
Fluen. 3 g a.i./L	8.7 a	8.5 a	6.0 bcd	8.3 a	6.8 c	6.7 b	7.8 b	8.7 a
Fluen. 6 g a.i./L	8.7 a	8.8 a	5.6 d	9.2 a	6.8 c	6.8 b	7.8 b	8.5 a
Fluen. 12 g a.i./L	8.8 a	8.8 a	5.8 cd	8.7 a	2.8 d	6.5 b	6.2 c	8.0 a
Oxamyl 4.8 g a.i./L	8.7 a	9.0 a	8.3 ab	8.7 a	7.8 bc	7.8 a	9.2 a	8.5 a
Untreated Check	9.0 a	8.2 a	8.0 abc	8.3 a	8.7 ab	7.8 a	9.2 a	8.8 a
Non-inoculated	8.8 a	7.7 a	9.2 a	8.7 a	9.5 a	8.2 a	8.7 a	8.5 a

[y]Vigor was conducted on a 0-10 scale with 0 being a dead plant and 10 being a live vigorous healthy plant

[z]Means are compared within columns. Means with the same letter do not significantly differ using Fisher's Protected LSD ($P \leq 0.10$).

Table 4.2 Effect of foliar applied nematicide on plant height[y] over 2 trials

Treatment	Squash 1	Squash 2	Cucumber 1	Cucumber 2	Eggplant 1	Eggplant 2	Tomato 1	Tomato 2
Fluen. 3 g a.i./L	13.5 a[z]	13.5 a	4.8 a	6.8 a	8.6 b	10.2 a	10.4 b	20.5 a
Fluen. 6 g a.i./L	13.5 a	13.7 a	4.8 a	6.3 a	8.1 b	10.2 a	12.4 a	18.8 a
Fluen. 12 g a.i./L	13.5 a	13.2 a	5.3 a	6.9 a	5.8 c	10.2 a	11.7 a	18.8 a
Oxamyl 4.8 g a.i./L	13.0 a	12.4 a	6.0 a	7.1 a	9.1 ab	11.4 a	11.7 a	18.5 a
Untreated Check	12.2 a	12.4 a	5.8 a	6.4 a	8.9 ab	11.9 a	11.9 a	18.5 a
Non-inoculated	12.7 a	11.9 a	6.0 a	6.6 a	10.2 a	11.7 a	11.9 a	19.1 a

[y]Plant heights are reported in centimeters 12 d after treatment

[z]Means are compared within columns. Means with the same letter do not significantly differ using Fisher's Protected LSD ($P \leq 0.10$).

Table 4.3 Effect of foliar applied nematicide on plant dry weight[y] over 2 trials

Treatment	Squash 1	Squash 2	Cucumber 1	Cucumber 2	Eggplant 1	Eggplant 2	Tomato 1	Tomato 2
Fluen. 3 g a.i./L	1.35 a[z]	0.70 a	0.58 b	0.73 a	1.05 b	0.90 a	1.80 c	1.60 a
Fluen. 6 g a.i./L	1.25 a	0.68 a	0.57 b	0.72 a	1.1 b	0.90 a	2.10 ab	1.50 a
Fluen. 12 g a.i./L	1.34 a	0.66 a	0.53 b	0.78 a	0.40 c	0.80 a	1.49 d	1.60 a
Oxamyl 4.8 g a.i./L	1.30 a	0.70 a	0.6 b	0.59 a	1.10 b	1.10 a	1.90 bc	1.30 a
Untreated Check	1.23 a	0.62 a	0.73 b	0.58 a	1.30 ab	1.10 a	1.80 c	1.80 a
Non-inoculated	1.47 a	0.68 a	1.21 a	0.62 a	1.65 a	1.30 a	2.31 a	1.70 a

[y]Plant dry weights are reported in grams

[z] Means are compared within columns. Means with the same letter do not significantly differ using Fisher's Protected LSD ($P \leq 0.10$).

Table 4.4 Effect of foliar applied nematicide on the number of *Meloidogyne incognita* females within the root[y] over 2 trials

Treatment	Squash 1	Squash 2	Cucumber 1	Cucumber 2	Eggplant 1	Eggplant 2
Fluen. 3 g a.i./L	896[z]	289	708	327	563	323
Fluen. 6 g a.i./L	873	330	740	385	663	295
Fluen. 12 g a.i./L	635	292	812	339	353	303
Oxamyl 4.8 g a.i./L	646	220	882	327	254	262
Untreated Check	822	342	881	399	496	349

[y]Number of female *M. incognita* nematodes counted within the stained root system

[z]Means are not significantly different within columns using Fisher's Protected LSD ($P \leq 0.10$)

Table 4.5 Effect of foliar applied nematicide on the number of *Meloigodyne incognita* galls within the root[y] over 2 trials

Treatment	Squash 1	Squash 2	Cucumber 1	Cucumber 2	Eggplant 1	Eggplant 2
Fluen. 3 g a.i./L	594[z]	199	419	259	355	209
Fluen. 6 g a.i./L	599	230	528	303	372	189
Fluen. 12 g a.i./L	505	209	466	273	194	249
Oxamyl 4.8 g a.i./L	493	162	538	266	170	163
Untreated Check	495	232	491	307	329	214

[y]Number of galls counted within the stained root system

[z]Means are not significantly different within columns using Fisher's Protected LSD ($P \leq 0.10$)

Figures

Figure 4.1 Photograph of transplants prior to nematicide treatment representing how the soil surface was covered to prevent nematicide contact with the soil.

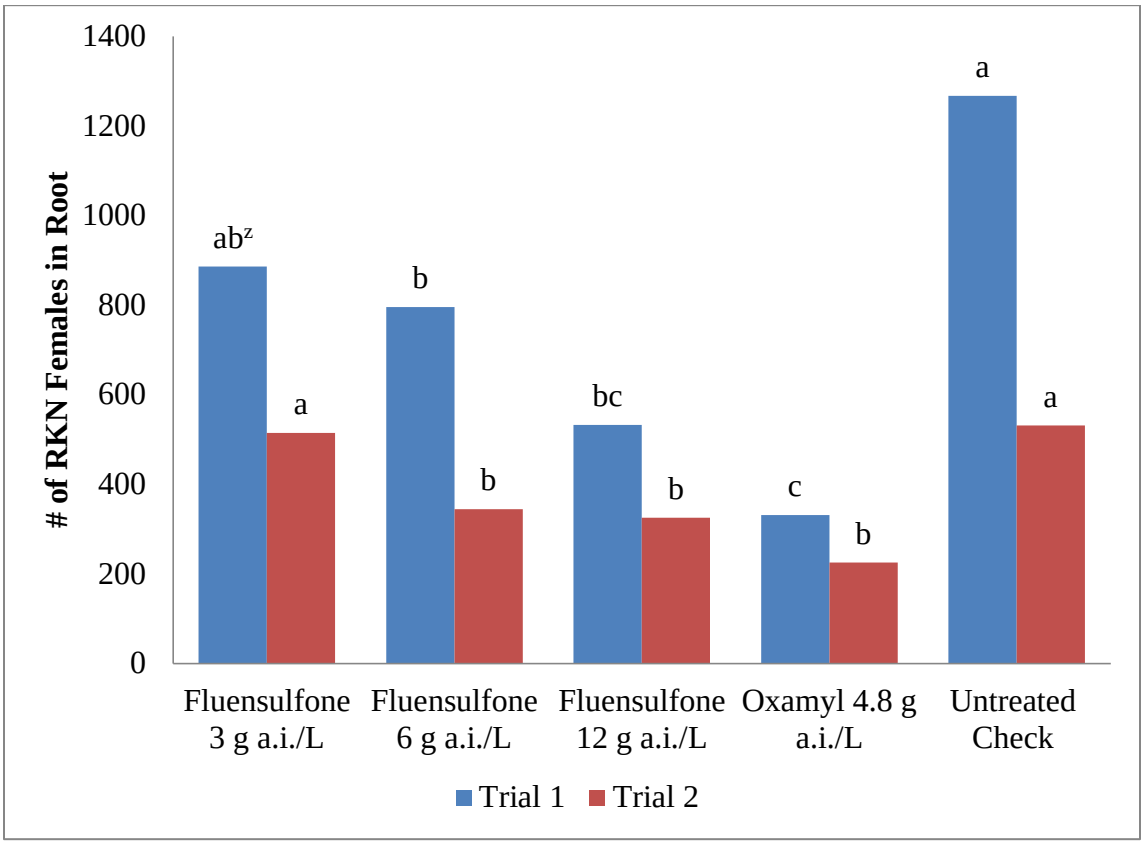

Figure 4.2 Nematicidal activity of foliar applied fluensulfone and oxamyl on the number of *Meloidogyne incognita* females found in tomato roots.
[z]Means within a trial with different letters are significantly different according to Fisher's Protected LSD ($P \leq 0.10$)

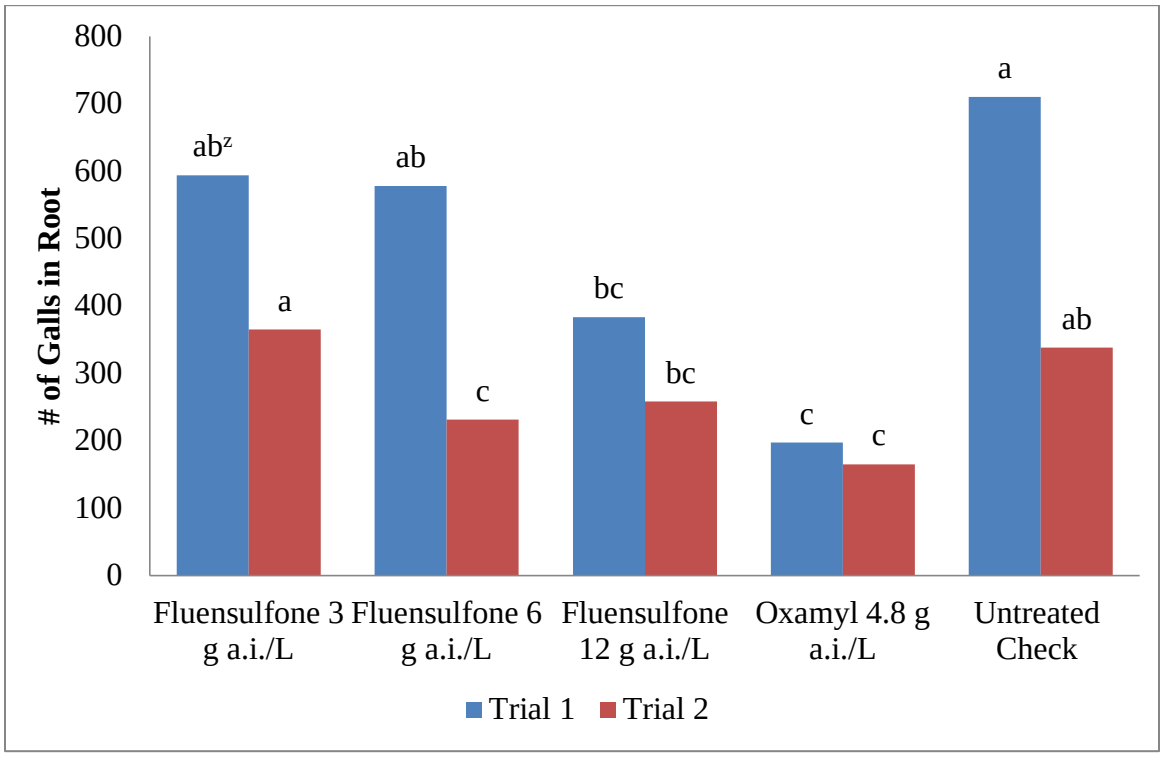

Figure 4.3 Nematicidal activity of foliar applied fluensulfone and oxamyl on the number of *Meloidogyne incognita* galls found in tomato roots.
[z]Means within a trial with different letters are significantly different according to Fisher's Protected LSD ($P \leq 0.10$)

CHAPTER 5

INVESTIGATION OF THE POTENTIAL DISEASE COMPLEX BETWEEN *PYTHIUM APHANIDERMATUM* AND *MELOIDOGYNE INCOGNITA* ON CUCUMBER

[1]Morris, K.A., D.B. Langston, R.F. Davis, P. Timper, J.P. Noe, and D.W. Dickson. To be submitted to *Plant Health Management.*

Abstract

A growth chamber trial and a field trial in 2012 demonstrated a possible disease complex between *Pythium aphanidermatum* and *Meloidogyne incognita* on cucumber. Two growth chamber trials were conducted in 2014 to investigate this potential disease complex. Treatments included cucumber seedlings inoculated with *P. aphanidermatum* alone, *M. incognita* alone, *P. aphanidermatum* + *M. incognita*, and an untreated control. Each pathogen was added to 14-day-old cucumber seedlings that were planted into 5 cm x 25 cm cone-tainers containing a 3:3:1 mixture of sand, sterilized field soil, and germinating mix, respectively. Inoculated pots received 1500 J2 stage *M. incognita* juveniles and/or 3000 g of sand-corn meal mix containing *P. aphanidermatum* inoculum. Once inoculated, cucumbers were placed in a growth chamber at 28^{o}C, 12 h photoperiod, and 75 % relative humidity. Plants were monitored daily for 3 weeks for symptoms of *P. aphanidermatum* infection. The presence of *P. aphanidermatum* was determined on symptomatic plants by isolating hypocotyl sections onto potato dextrose agar (PDA) and observing the colony morphology and microscopic characteristics of *P. aphanidermatum*. In both trials, a significant statistical synergistic interaction occurred when pots were inoculated with both pathogens than pots inoculated with either pathogen alone (P=0.015 for trial 1 and P=0.0002 for trial 2). These results indicate that a disease complex exists between *M. incognita* and *P. aphanidermatum* on cucumber.

Introduction

Diseases complexes involving plant-pathogenic nematodes and soil-borne fungi have been documented for more than 100 years. Atkinson (1892) first described a disease complex on cotton involving *Meloidogyne incognita* and *Fusarium oxysporum*. Since

then, a wide variety of disease complexes have been described over a range of crops (Back *et al.*, 2002). Many of these interactions involve *Meloidogyne* spp., or root-knot nematodes, which cause extensive root galling on host plants that can lead to chlorosis, stunting, wilting, and ultimately yield reduction due to the lack of uptake of water and nutrients. Infection by *Meloidogyne* spp. may predispose plants to secondary pathogens or pathogens that otherwise may not be as virulent by themselves. *Pythium* spp. are known to interact synergistically with *Meloidogyne* spp., causing disease complexes on tobacco Melendez and Powell, 1970 and chili pepper (Hasan, 1985). Synergistic disease interactions are characterized by the extent of plant damage when nematodes and fungi are found together in the soil being greater than the sum of the damage that would incur from either pathogen alone (Back *et al*., 2002).

Most disease complex studies have been conducted on field crops such as cotton, bean, soybean, and tobacco (Batten and Powell, 1971; Rupe, 1989; France and Abawi, 1994; Nakajima *et al*., 1996; De Vay *et al.*, 1997; and Abd-El-Alim *et al.*, 1999). Conversely, there have not been as many disease complex studies on vegetable crops. Suleman *et al.* (1997) described a disease complex between *M. incognita* and *Fusarium oxysporum* f. sp. *lycopersici* of tomato where the presence of both pathogens caused an increase in Fusarium wilt symptoms. A similar interaction occurs on tomato with *Rhizoctonia solani* and *M. incognita* (Arya and Saxena 1999). Currently, there are no nematode-soil borne pathogen disease complexes reported on cucumber (*Cucumis sativus*) or other cucurbits.

A growth chamber study in 2012 suggested a significant increase in cucumber seedling damping off due to *Pythium* spp. in pots inoculated with *M. incognita* compared

to pots which were not inoculated with *M. incognita* (Fig. 5.1). That same year, a high incidence of Pythium damping off was observed in a cucumber nematicide field trial. Plots which received a nematicide treatment had significantly higher stand counts than plots which did not receive a nematicide and plant death was attributed to *Pythium* spp. (Fig 5.2). These two observations prompted the investigation of a potential disease complex in cucumber between these two pathogens. The objective of this study was to determine whether a synergistic disease interaction exists between *M. incognita* and *P. aphanidermatum* in cucumber.

Materials and Methods

Identification of Pythium species: A *Pythium* sp. was isolated from an infected cucumber seedling that had been sent to the University of Georgia Tifton Plant Disease Clinic. The isolate was grown on potato dextrose agar (PDA) for 3 d. An 8-mm agar plug was placed into a petri dish containing distilled water to stimulate the development of reproductive structures. Microscopic examination (400 x) revealed sporangia with morphological characteristics similar to *P. aphanidermatum.* The isolate was subjected to conventional PCR using *cox II* gene primers (Martin, 2000) which are specific to *Pythium* spp. DNA amplification was conducted using the method described by Villa, *et al.* (2006). A positive identification band on agarose gel indicated that the presence of PCR products were a *Pythium* spp. The isolate was sent to Eurofins Genomics (Huntsville, AL) for sequencing. Sequencing revealed 99% homology with *P. aphanidermatum.*

Pythium inoculum: *Pythium aphanidermatum* inoculum was prepared using the sand cornmeal inoculum method (Kirkpatrick, 2012). Six hundred ml of sand and 50 ml of cornmeal were mixed with 240 ml of distilled water in a 2000 ml polypropylene flask.

Nine flasks were autoclaved at 120 C for 40 m and then allowed to rest for 24 hr before being autoclaved again at 120^{o}C for 40 min. *Pythium aphanidermatum* was grown on PDA for 4 d, then 10 8-mm-diameter agar plugs were taken from the actively growing regions of *Pythium* cultures and placed in each flask containing the sand-cornmeal mixture. The inoculum was allowed to colonize the flask for 9 d. Each flask was gently shaken every 2 d to insure even distribution of inoculum within the flask.

Meloidogyne incognita inoculum: Eggplant ('Black Beauty') were inoculated with *M. incognita* and were allowed to grow for 90 days before roots were excised and placed on hardware cloth over a large pan in a mist chamber to collect second-stage juvenile (J2) nematodes. Roots remained in the mist chamber for 7 d. Every 2 d, the water collected in the pans and passed through nested 200 and 500 mesh seive. Juvenile nematodes were then washed from the 500 mesh sieve and counted with a dissecting scope (40 x). Juvenile nematodes were then stored in a refrigerator for 7 d at 4.4 ^{o}C until the time of inoculation.

Growth chamber cone-tainer studies: Treatments consisted of *P. aphanidermatum* alone, *M. incognita* alone, *P. aphanidermatum* + *M. incognita*, and an untreated control. A soil medium consisting of 3:3:1 coarse sand, pasteurized field soil, and a germinating mix, respectively, were used in these experiments. Soil was homogenized in a 30 gallon cement mixer. Water was added to the mix until a consistency favorable for seedling growth was achieved and then placed into 5-cm x 25-cm cone-tainers. Treatments that did not receive a *P. aphanidermatum* treatment were mixed first to reduce possible contamination. The *Pythium* inoculum was added to the soil in the cement mixer at a rate of 3000 g per batch which would yield ~100 g of *P.*

aphanidermatum inoculum per cone-tainer. Nine-day-old 'Rockingham' cucumber seedlings were transplanted into each cone-tainer. Nematode inoculum was pipetted evenly around each cucumber transplant at 1,500 J2s per cone-tainer. Plants were placed in a growth chamber for 3 weeks at 28 ^{o}C, 75% relative humidity, and a 12 hr photoperiod for 3 weeks. Pots were arranged in a completely randomized design with 4 replications with 10 plants per replication for a total of 40 plants per treatment. The experiment was conducted twice.

Data collection and statistical analysis: Plants were monitored daily for symptoms of *P. aphanidermatum* infection. Once symptoms were observed, infected plants were taken to the laboratory and *P. aphanidermatum* was isolated by placing hypocotyl sections onto PDA and observing the colony morphology and microscopic characteristics of *P. aphanidermatum.* Final disease incidence was recorded from the percentage of the 10 plants per replication that were infected with *Pythium*. In the second experiment, plant vigor was assessed using a 0 to 10 visual scale with 0 being a dead plant and 10 being a live vigorous healthy plant. Plant vigor data was subjected to Fisher's test of LSD ($P \leq 0.01$) using ARM statistical software (Gylling Data Management, Brookings, SD). Disease incidence data was evaluated using factorial analysis of variance (GLIMMIX) procedure in SAS (SAS Institute, Cary, NC) to determine if a significant synergistic interaction occurs when both pathogens are present.

Results

Disease incidence: In both experiments, pots which contained both *P. aphanidermatum* and *M. incognita* demonstrated significantly greater damping-off from *P. aphanidermatum* than any other treatment at $P \leq 0.01$ (Table 5.1). A higher percentage

of plants damped-off from *P. aphanidermatum* infection in trial 2 than in trial 1. In the first experiment, 16% of plants inoculated with both *P. aphanidermatum* and *M. incognita* died, while no plants died in the other treatments. In the second experiment, 68% of plants inoculated with both pathogens died versus 2.5% with *P. aphanidermatum* alone, 5% with *M. incognita* alone, and 0% in the untreated. A significant interaction exists between trials (P=0.0007) due to the differences in magnitude of plant death recorded in each trial; however, a factorial ANOVA analysis revealed a significant synergistic interaction when both pathogen are present (Table 5.1) in each respective trial (Trial 1 P=0.015, Trial 2 P=0.0002).

Vigor data: Vigor data collected on surviving plants in the second experiment revealed that there was a decline ($P \leq 0.01$) in plant vigor when both pathogens were present versus when either one occurs alone (Fig. 5.3). A visual vigor difference was not noted in the first experiment and therefore vigor data was not collected.

Discussion

In both trials, greater numbers of seedlings died from *Pythium* damping-off in treatments where both pathogens were inoculated to cucumber seedlings compared to either pathogen alone. These data indicate a synergistic disease interaction between *P. aphanidermatum* and *M. incognita* on cucumber. In trial 2, plants appeared chlorotic and stunted, indicating that even if *P. aphanidermatum* did not cause damping off there could still be a dramatic decline in yield when these two pathogens are found together. This reduction in vigor was not observed in the first experiment. Likewise, there was significantly less total plant death in the first experiment compared to the second experiment. This could be due to a variety of factors. It could be that the nematode

inoculum in trial 2 was more viable and led to greater nematode infection. However, galling indices were not collected in these trials so this cannot be proven. The amount or presence of RKN in soil may determine whether or not there will be increased stand loss due to *Pythium* damping off since very few plants died when *Pythium* was the only pathogen present. These ideas are reinforced by the observations made in 2012 when an increase in seedling death was noted in pots inoculated with *M. incognita* versus non-inoculated. Moreover, in the field trials an efficacious nematicide reduced the incidence of *Pythium* damping off. Another possibility for the increase in seedling death in the second experiment is soil moisture, which was not measured. Since *Pythium* is an oomycete, water is required for zoospores to actively swim through the soil solution and penetrate the root. An inadvertent increase in soil moisture could have made for a more favorable environment for *P. aphanidermatum.* Five percent of plants infected with only *M. incognita* alone died in the second experiment from *Pythium* damping off. This could be due to cross contamination either while the soil was being mixed or during an irrigation event that splashed *Pythium* inoculum into these pots, or it could be due to incomplete sterilization of soil media.

Because *Meloidogyne* spp. are obligate parasites, it could potentially be detrimental to the nematode population if there is an increase in seedling death when they occur simultaneously with *Pythium* spp. In both experiments, seedling death occurred within 10 days. *Meloidogyne* spp. typically take about 28 days to reproduce depending on soil temperature (Ploeg and Maris, 1999). Therefore, *Meloidogyne* populations would not have enough time for a new generation to be produced and populations could ultimately decline depending on the extent of plant death. However, this idea contradicts

the findings of Hasan (1985) where an increase in *M. incognita* reproduction was observed on chili plants infected with *Rhizoctonia solani* and *Pythium aphanidermatum*.

Control of seedling diseases in cucumber is heavily reliant upon pesticides. Mefenoxam and metalaxyl are commonly applied to cucumber to control soil-borne oomycetes diseases. Because our study documents a disease complex, control of *Meloidogyne* spp. may also be important for controlling *Pythium* damping-off in cucumber. Studies that combine fungicides and nematicides would help elucidate management options for this disease complex. The mechanism by which these interactions occur is not clearly understood. Mechanical damage caused by nematodes has been reported as influential in the establishment of fungal pathogens within a root (Inagaki and Powell, 1969). However, other studies found that mechanical damage does not influence disease interactions (Taylor, 1990). *Meloidogyne* spp. have intimate relationships with their respective hosts and the establishment of giant cells creates an area in the root of high metabolic activity. The cells within these specialized feeding structures have an unusually high number of organelles (Jones, 1981). It has been proposed that these "nutrient sinks" may serve as a suitable substrate for fungal colonization (McLean and Lawernce, 1993; Abdel-Momen and Starr, 1998). A modification of the rhizosphere (Back et al., 2002) is another potential mechanism by which disease interactions involving nematodes may occur as an infection by plant-parasitic nematodes may stimulate the release of root exudates that attract soil-borne fungi (Bergeson, 1972). In addition, *P. myriotylum* was the *Pythium* spp. isolated from the 2012 growth chamber trial; therefore, it is unlikely that this disease complex is limited strictly to *P. aphanidermatum*, but could potentially include other *Pythium* spp. as

well. The mechanism of the disease interaction between *M. incognita* and *Pythium* spp. is unknown and studies could be conducted to determine how the interaction occurs. In addition, inoculum levels of each pathogen necessary to induce the interaction need to be determined.

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Tables

Table 5.1 Percent plant death caused by *Pythium* damping-off of cucumber when *Pythium aphanidermatum* and *Meloidogyne incognita* occur simultaneously in soil

Treatment	Trial 1	Trial 2
RKN	0.0 b[z]	7.5 b
Pythium	0.0 b	2.5 b
RKN + Pythium	15.0 a	70.0 a
Non-inoculated	0.0 b	0.0 a
RKN x Pythium Interaction *P*=	0.015	0.0002

[z] Means with the same letter do not significantly differ according to Fisher's Protected LSD ($P \leq 0.01$).

Figures

Fig 5.1 Photograph of seedling death caused by *Pythium myriotylum* in a greenhouse study in 2012. Plants that are dead were inoculated with *M. incognita* and alive plants were not.

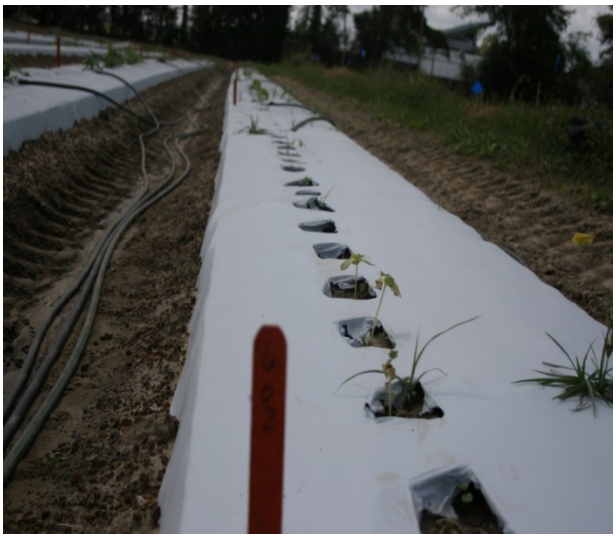

Fig. 5.2 Photograph of plots treated with a nematicide (left) and untreated (right). Plant death is a result of *Pythium* damping off.

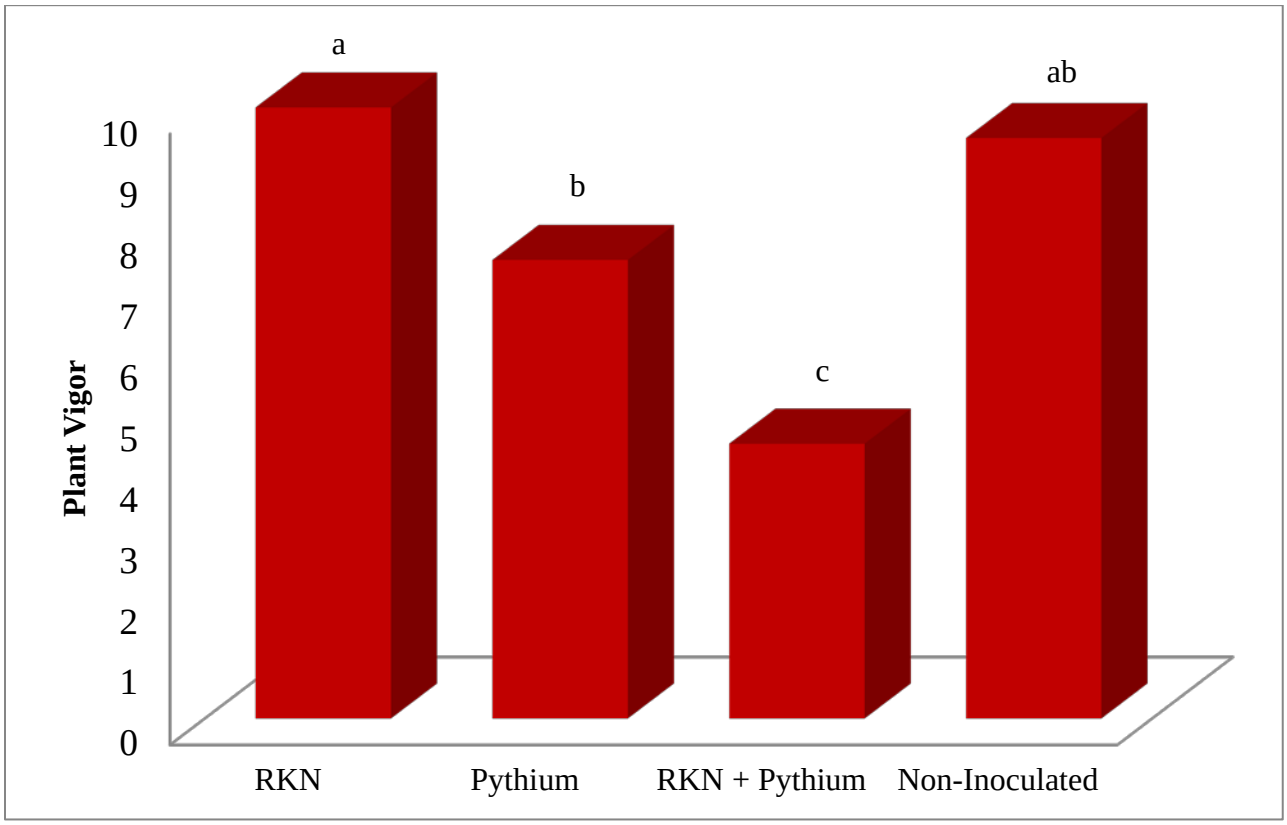

Fig. 5.3 Plant vigor in experiment two where 0=completely dead plants and 10=plants that are completely healthy.
[z] Means with the same letter do not significantly differ according to Fisher's Protected LSD ($P \leq 0.01$).

CHAPTER 6

SORPTION AND MOBILITY OF FLUENSULFONE ON VARIOUS SOILS

[1]Morris, K.A., D.B. Langston, R.F. Davis, P. Timper, J.P. Noe, D.W. Dickson, T. Grey, and S. Li. To be submitted to *Pest Management Science*

Abstract

Fluensulfone is a new novel nematicide in the fluoroalkenyl chemical class which recently received Federal EPA registration. The adsorption/desorption of fluensulfone was tested on 9 soils from various regions of the U.S. and mobility experiments were conducted on 4 soils. Adsorption data was fitted to the logarithmic form of the Freundlich isotherms (K_F). K_F values varied from 1.24 to 3.28. Desorption of fluensulfone was evaluated for 5 concentrations on 9 soils. The Arredondo sand had desorption rate of 26% while the remaining soils ranged from 43 to 70% at a concentration of 40 µMol/L. Soil parameters were subjected to Pearson's correlation test. Adsorption of fluensulfone was positively correlated with organic matter content (0.666), silt (0.545), and clay (0.340) and negatively correlated with sand fraction (-0.544). Desorption was correlated to pH (0.382) and cation exchange capacity (0.444) of the soil. In the first mobility experiment, fluensulfone was applied to columns (10 x 38 cm) filled with Arredondo sand at a concentration of 2.43 mg/ml, and then flushed with 4000 ml of water per column. Leachate was collected from the bottom of the column hourly for 9 hours. Fluensulfone concentration in the leachate peaked 3 hours after initiation and then gradually declined, becoming undetectable after 9 hours. Recovery from leachate was 45% of the initial fluensulfone applied to the column. The second mobility experiments evaluated the location of fluensulfone within the columns on 4 soils. Columns were treated with fluensulfone and then 1000 ml of water was drip applied to the top of the soil. Leachate was collected from the column, then columns were cut into three 10 cm sections once the trial was completed. Fluensulfone was least mobile in Chualar sandy loam with 41% of recovered pesticide located in the top 10 cm

of soil, followed by the Arredondo sand (34%), Greenville sandy clay loam (29%), and Tifton loamy sand (13%). These results indicate that organic matter and clay content of soils can be determining factors for adsorption and that the mobility of fluensulfone varies on different soils.

Introduction

The ban of methyl bromide (MeBr) triggered an increase in research of MeBr alternatives. Some research objectives have focused on the use of non-fumigant pesticides as potential candidates for MeBr replacements. Fluensulfone is a non-fumigant nematicide that is in the fluoroalkenyl chemical class with an unknown mode of action differing from traditional non-fumigant organophosphates and carbamates (Kearn *et al.*, 2014). Fluensulfone received EPA registration in September 2014 under the trade name NIMITZ™ (ADAMA Agricultural Solutions Ltd. Raleigh, NC). Reports on the efficacy of fluensulfone against *Meloidogyne* spp. have shown it to be viable nematicide (Oka et al., 2012; Csinos *et al.*, 2010, Oka *et al*., 2009). However, there is no published data concerning the fate of fluensulfone in soil after field application.

The sorption potential of fluensulfone to various soils and its effect on the mobility has not been reported. The sorption of pesticides to soil particles makes them unavailable in the soil solution and can reduce their efficacy against target species. This is an important concept for contact nematicides because the target species are in the soil solution; therefore, effective nematicide concentrations must be soil solution for adequate control (Smelt and Leistra, 1992). In addition to reducing efficacy, pesticide adsorption affects mobility throughout the soil profile, which can impact its influence on the environment (Karpouzas *et al*., 2007). The sorption of other non-fumigant nematicides

has been considerably researched with many studies finding a strong correlation between sorption and soil properties (Pantelelis *et al.*, 2006; Qin *et al*, 2004; Simon *et al*., 1992; Bilkert and Roa, 1985; Gerstl, 1984). A bioassay conducted by Oka *et al.* (2013) reported that the addition of organic matter (O.M.) to soils significantly reduces the efficacy of fluensulfone against *Meloidogyne javanica* as well as significantly reducing mobility in the soil profile. In the same study, Oka *et al.* (2013) noted that fluensulfone mobility was inhibited by soils with greater clay content. These results indicate that fluensulfone has an affinity to bind to O.M. and may be mobile through soils with a high sand content.

The initial fluensulfone registration is targeted at fruiting vegetables and cucurbits. The majority of these crops are grown in the southeast U.S. and in California. Soils common to the southeastern U.S. generally have low O.M. content. Therefore, these soils may not inhibit fluensulfone mobility. Also, these soils generally have a high sand fraction which may allow percolation. In these studies the adsorption and desorption of fluensulfone was tested on 9 soil types. In addition, the mobility of fluensulfone was evaluated on 4 soils.

Materials and Methods

Nematicide: Technical grade fluensulfone (97.56% purity) was supplied by ADAMA Agricultural Solutions Ltd. (Raleigh, NC). Fluensulfone (5-chloro-2-(3,4,4-trifluorobut-3-enylsulfonyl)-1,3-thiazole) structural composition is shown in Fig. 6.1. The technical grade fluensulfone was used to determine the timing of a fluensulfone peak when subjected to HPLC analysis. Adsorption-desorption and mobility experiments were

conducted using a formulated emulsified concentrate of fluensulfone at 480 g a.i./L. All experiments were completed once with 6 replications.

Adsorption-desorption experiments: Adsorption experiments were conducted on 9 soil types. The top 20 cm of each soil were collected and screened over a 4.7 mm sieve to remove rocks and debris from the samples. Soil origin, type, taxonomy, texture, CEC, organic matter content, and pH are shown in Table 6.1. Since the initial EPA registration for fluensulfone is labeled for vegetables, the Florida, Georgia, and California soils were chosen in these experiments because of the extensive vegetable production in these regions. The remaining soils (Kentucky, Colorado, and Texas) were chosen because of the distinctly different properties they have compared to traditional vegetable growing areas.

Ten gram samples of dried, sifted soil were placed into 50 ml polypropylene centrifuge tubes. Twenty ml of 0.01 $CaCl_2$ solution containing concentrations of 1, 5, 10, 20 and 40 µmol/ml of fluensulfone was added to each centrifuge tube. Preliminary experiments indicate that fluensulfone did not bind to the polypropylene tubes (data not shown). Formulated product of fluensulfone containing 480 g of active ingredient per L was used to prepare the $CaCl_2$ solution. Samples were placed on a reciprocal shaker at 22 ^{o}C for 24 h in order to reach equilibrium (Norshie, 2014 unpublished). Samples were then set aside for 10 minutes to allow sediment to settle. A 2 ml sample of the supernatant was centrifuged (Eppendorf MiniSpin, Eppendorf, Hamburg, Germany) at 13,000 rpm for 2 minutes, and the supernatant was then placed into 2 ml HPLC vials for analysis.

Quantification of fluensulfone was performed using a Waters 2695 HPLC and a Waters 2996 PDA detector. A Waters XTerra™ Shield RP18 column (4.6 mm x 150 mm, 5 µ m. Waters Co. Milford, MA) was used for separation. Column temperature was set at 60 ^{o}C. Distilled water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B) was used as the two mobile phases. Total sample run time was 15 minutes. The flow rate was set for 0.5 ml/min at a detection wave length of 280 nm. The flow program consisted of 85% and 15% of mobile phase A and B, respectively, for the first 12 minutes. The flow gradient was changed to 10% A and 90% B from 12-13 minutes before reverting back to 85% A and 15% B for the final 2 minutes. Fluensulfone eluted at 12.05 minutes without interference. The difference between the initial concentration of fluensulfone and the observed concentration at equilibrium was used to determine the amount of soil adsorbed fluensulfone.

Adsorption data was fitted to the logarithmic form of the Freundlich isotherms (Jaroniec, 1975):

$$\log C_S = \log K_F + 1/n \log C_E \qquad [1]$$

where *Cs* is the amount of fluensulfone adsorbed to the soil at an equilibrium concentration (C_E). K_F is a constant term that characterizes the sorption capacity of a soil while 1/*n* is a constant that characterizes the sorption intensity. K_F is a mathematical description of the distribution of a pesticide between the solid and solution phases. The higher the K_F value, the more tightly a compound is bound to the soil particles. Sorption of pesticides is largely related to organic matter and the percentage of organic matter varies among soils (Jury *et al.*, 1983). Therefore, sorption data was normalized using K_{FOC} with OC being soil organic carbon using the formula:

$$K_{FOC}=K_F/OC \times 100 \quad [2]$$

Organic carbon is calculated by multiplying the percent organic matter of a soil by 0.56 which is the estimated percentage of organic matter which is actually carbon. K_D values are a measure of how tightly the pesticide binds to soil particles. The greater the K_D value, the less likely a chemical will be mobile or contribute to runoff. According the EPA, a pesticide with a K_D value >5 has extremely low leaching potential. K_D values were calculated using the equation:

$$K_D=C_S*10/C_E*20 \quad [3]$$

where 10 is the amount of treated soil in grams and 20 is the amount of $CaCl_2$ solution added to each sample in ml.

Desorption experiments of fluensulfone were conducted after adsorption experiments. The initial supernatant from the adsorption studies was decanted and replaced with 20 ml of 0.01 µmol/L $CaCl_2$. Samples were shaken for 24 h at 22 ^oC, were centrifuge as described above, and subjected to HPLC analysis with identical methods used for quantification of fluensulfone with adsorption studies. The percentage of fluensulfone that desorbed from the soil was calculated using the equation:

$$\text{Desorption}=C_E*20/C_D*10 \quad [4]$$

where C_E is the detection concentration and 20 is the amount in ml of $CaCl_2$ solution added to each sample and C_E is the equilibrium concentration times 10 g of soil. Data obtained from these experiments was combined.

Mobility experiments: Mobility experiments were conducted on the Arredondo sand soil to quantify how much fluensulfone could be flushed through a column with

large irrigation volume. Polyvinyl chloride (PVC) columns (10 cm diameter) were used in these experiments. Columns were cut into 38-cm-long sections and a polypropylene plastic plug was glued onto one end of the columns. Five 1-cm holes were drilled into the bottom of the plug to allow leachate to pass through. A 2.5-cm layer of steel wool was inserted into the bottom of the columns to help maintain soil integrity. The column was then filled with 30.5 cm of Arredondo sand soil. The columns were suspended ~15 cm above the ground via a rope tied to a steel rack and water was passed through the column until saturation occurred and leachate was observed dripping from the bottom. The column was allowed to rest for 24 hr to allow time for the soil to reach field capacity prior to the application of fluensulfone. Fluensulfone (2.43 mg) was mixed with 10 ml of water and then applied to 50 g of soil which were then incubated for 1 hr to allow time for fluensulfone adsorption to the soil. For each column, 50 g of treated soil was applied to the top of the soil in the columns and then covered with 2.5 cm of silicate sand. The silicate sand was used to disperse the irrigation added to the column in an even manner.

A 1000 ml polypropylene beaker was placed underneath the suspended column to catch the leachate. A 500 ml separation funnel was suspended over the top of the soil in the columns. The valve on the funnels was set to allow ~0.2 ml of water/second to drip on the soil surface. A total of 4000 ml of water was allowed to pass through the columns via the separation funnels. Seven hours of time elapsed before the 4000 ml had been applied. The volume of leachate collected at each collection point was recorded. Samples were then centrifuged and subjected to HPLC analysis in the same manner and protocol as described for the adsorption-desorption experiments. The amount of

fluensulfone that was detected in the leachate was compared to the known initial concentration to achieve a total recovery percentage of fluensulfone.

The mobility of fluensulfone was evaluated on Greenville sandy clay loam (Plains, GA), Tifton loamy sand (Tifton, GA), Arredondo sand (Citra, FL) and Chualar sandy loam (Salinas, CA) soils with limited irrigation. Trial preparation was identical to the mobility experiment described above. A total of 1000 ml of water was applied to each column. The total volume of leachate that passed through each column was measured, recorded, and subjected to HPLC analysis as previously described. The columns were incubated for 2 hr after the 1000 ml irrigation event was over to insure that they reached field capacity again. The columns were then placed in a refrigerator at 4.4 C to prevent microbial degradation of fluensulfone until the columns could be cut into sections and its contents analyzed. The columns were taken out of the refrigerator and were measured into three 10 cm sections. These sections were then cut with a band saw and the soil from each section was placed in a plastic Ziploc® bag. A 50 g representative sample from each soil core was taken and put in a 250 ml flask and mixed with 25 ml of distilled water plus 25 ml of acetonitrile. The flasks were placed on a rotary shaker for 3 h then allowed to rest for 10 minutes to allow for sediment to settle to the bottom. A 2 ml sample of the supernatant was centrifuged and subjected to HPLC analysis as previously described. The amount of fluensulfone that was detected in the leachate and soil samples was compared to the known initial concentration to achieve a total recovery percentage of fluensulfone and its location within the column.

Statistical analysis: Pearson correlations were conducted using SAS 9.3 (SAS Institute, Cary NC). Isotherms were made using Sigmaplot 12.0 (Systat Software, Inc. San Jose, CA).

Results

Adsorption-Desorption: K_F values from equation [1] ranged from 1.24 (Tifton loamy sand) to 3.28 (Sonora silt loam) (Table 6.2). However, the majority of the K_F values were between 1.0 and 2.0. All r^2 values were 0.93 or greater indicating that the Freundlich model was a good fit. K_{FOC} values from equation [2] ranged from 188 (Haxton sandy loam) to 1075 (Arredondo sand). The greater the K_{FOC} value the more tightly fluensulfone is bound to the soil.

K_Ds from equation [3] were calculated for 5 different concentrations of fluensulfone on each soil type (Table 6.3). The greater the K_D value the more tightly the pesticide is bound to the soil and the less mobility. K_Ds generally decreased as the concentration of fluensulfone increased. The values ranged from 0.73 (Tifton loamy sand) to 3.86 (Sonora silt loam) at a concentration of 1 µmol/L. These results are consistent with the K_F values in Table 6.2 with Tifton loamy sand and Sonora silt loam having the lowest and highest sorption intesities, respectively.

Adsorption isotherms are shown in Figure 6.2. At concentrations of 1, 5, and 10 µmol/L, soil adsorbed fluensulfone was difficult to distinguish among soil types. However, at higher concentrations of 20 and 40 µmol/L the Yolo silt loam, Tifton loamy sand, and Sonora silt loam had the greatest amount of adsorbed fluensulfone. The Tremona sand had the least amount of adsorbed fluensulfone when equilibrium concentrations increased.

Fluensulfone desorbed from the Haxton sandy loam at a far greater percentage at all rates than any other soil tested (Table 6.4). Over 80% of the initial concentration added desorbed from the sample at an equilibrium concentration of 10 and 20 µmol/L. The Arredondo sand had the least amount of fluensulfone desorbed at any concentration with as little as 9% of the initial concentration of 5 µmol/L being desorbed.

Pearson's correlation coefficients for each soil type were calculated and presented in Table 6.5. K_F was significantly positively correlated to OM (r^2=0.6664), silt (r^2=0.5450), and clay (r^2=.3407) and is negatively correlated to sand content (r^2=-0.5447). The desorption of fluensulfone was not correlated to the same parameters as K_F. Desorption has a positive correlation with pH (r^2=.3825) and cation exchange capacity, or CEC (r^2=0.4442). All data were significant at $P \leq 0.05$.

Mobility Experiment: The Arredondo sand was chosen for the mobility experiment when a large volume of irrigation was used because of the high sand fraction associated with this soil type. Peak collection of fluensulfone in the leachate occurred at the 3 hr sample time and declined with each subsequent collection time (Figure 6.3). Fluensulfone was not detected at the last collection time of 9 hr. A total of 45% of the initial concentration applied to the columns was recovered after an irrigation event of 4000 ml was applied.

For the second mobility experiments the Greenville sandy clay loam had the greatest overall recovery percentage at 96% (Figure 6.4). This soil also had an even concentration distribution of fluensulfone at the 3 depths analyzed in the column. However, the Tifton loamy sand (Figure 6.5) had the most evenly distributed amounts of fluensulfone throughout the column and leachate with 13%, 16%, 23%, and 21%

recovered at the 4 respective collection sites. A total of 73% of the initial concentration applied to the Tifton loamy sand was recovered. The Arredondo sand (Fig 6.6) showed a gradual decrease in the concentration of fluensulfone. A total of 34% of the recovered fluensulfone in the Arredondo sand was still within the top 10.16 cm of soil after 1000 ml of irrigation. A total of 80% of the initial concentration was recovered from the Arredondo sand. Fluensulfone showed the least mobility on the Chualar sandy loam (Figure 6.7) with 41% of the total recovered remaining in the top 10.16 cm of soil. Only 10% of the total fluensulfone recovered was found at the deepest depth in the column (20.32-30.48 cm) and only 0.15% of the total recovered was found in the leachate. A total of 85% of the initial concentration was recovered from the Chualar sandy loam.

Discussion

The sorption of fluensulfone varied among the different soil types tested. These differences are predominately due to the amount of organic matter found in the respective soils. Since fluensulfone is a non-polar molecule it is not surprising that adsorption was primarily dependent on the amount of organic matter found in the soil (Calvet, 1989). Although sorption was variable across the soils tested all sorption levels were generally low indicating that fluensulfone has a low sorption capacity. If an overall small amount of fluensulfone applied to a soil is adsorbed, then a greater amount should be biologically available in the soil solution and therefore sorption should not affect the efficacy of the compound against nematodes.

The relatively low K_{FOC} values are another indication that fluensulfone sorption potential is generally low. It is surprising that the Arredondo sand and Tremona sand have the highest K_{FOC} considering that they have the lowest organic matter percentage

and the highest sand fraction of any soil tested. These results suggest that fluensulfone may have an affinity to bind to other compounds in the soil other than organic matter, silt, and clay. The possibility that these soils have a high concentration of iron oxide was not tested. Iron oxide has been reported as the most efficient adsorbent of hydrophobic compounds (Pierce *et al.*, 1971), and therefore could be a factor in the adsorption of fluensulfone to soils with low organic matter content. Iron oxide also could have contributed to the large K_D value that was noted with the Arredondo sand. Future studies should be conducted to attempt to elucidate the reason why fluensulfone has shown an affinity for binding to Arredondo sand.

K_D reported in this study demonstrated a dramatic decline once the concentration of fluensulfone reached 5 µMol/L, but the values differed little between the 5 µMol/L and the 40 µMol/L rate. However, differences in K_D when fluensulfone was applied at a low concentration of 1 µMol/L were more definitive. These results were expected with the Sonora silt loam, Greenville sandy clay loam, and the Yolo silt loam because these soils have either high organic matter content or a high percentage of clay and silt. Since K_D declined as concentrations of fluensulfone increased, it is likely that there are a limited number of binding sites available for fluensulfone to occupy. An increase in application rate has been documented to be inversely related to the amount of a pesticide bound to a soil (Gan *et al.*, 1995; Racke and Lichtenstein, 1987).

The mobility of a pesticide through soil is dependent upon their capacity and potential to sorb and desorb from soil particles (Arias-Estevez *et al.*, 2007; Moorman *et al.*, 2001). Since sorption of fluensulfone is largely correlated to soil organic matter content, it is no surprise that the Tifton loamy sand displayed the most even distribution

of fluensulfone since it has a relatively high sand fraction and low organic matter content. The lack of mobility through the Arredondo sand and the Chualar sandy loam is more difficult to explain since the soils have low organic matter content and low clay content. Water percolated through the packed soil columns filled with Chualar sandy loam at a much slower rate than any other soil tested. This is consistent with soil survey data describing a Chualar sandy loam as having moderately slow water permeability (USDA). The other soils evaluated in these tests have either moderate or rapid water permeability. This could help explain why >40% of the fluensulfone recovered was found within the top 10 cm of the Chualar sandy loam. The movement of pesticides through defined soil structures has shown that pesticides can move through heavy soil types at a faster rate than predicted due to a phenomenon known as preferential flow (Carter, 1999; Kladivko *et al.*, 1991; Steenhuis and Muck, 1988). Preferential flow is the uneven and rapid movement of water through soil due to cracks in the soil structure. However, despite widespread reports of the effect that preferential flow has on pesticide mobility, it was not noted in these trials. Ghodrati and Jury (1992) suggested that natural soil structure is destroyed under laboratory conditions and thus eliminates preferential flow. Therefore, fluensulfone may be more mobile in field studies but further research would need to be conducted to prove this.

Factors other than clay and organic matter content contributed to the lack of mobility through the Arredondo sand. These results contradict other nematicide mobility studies conducted on Arredondo sand. Bilkert and Rao (1985) found that aldicarb, a carbamate, rapidly leached through Arredondo sand. It was also surprising that fluensulfone mobility was evenly distributed throughout the Greenville sandy clay loam

considering that this soil had the highest organic matter and clay content of any soil tested. However, water did permeate through these columns at a steady rate which could have allowed fluensulfone to be desorbed from the soil at a more efficient rate than that of the Chualar sandy loam which could have resulted in increased mobility.

The sorption of fluensulfone did not vary greatly on the soils tested, especially when concentrations were > 5 µmol/L. The 20 µmol/L concentration is comparable to a field rate application of fluensulfone; therefore, adsorption of fluensulfone should not affect its bioavailability in the soil. Likewise, fluensulfone was mobile through at least the top 20 cm of soil on each soil type tested. Plants grow increasingly tolerant to nematode infection as they age (Freckman and Caswell, 1985). Therefore, the most critical time for a plant to be protected from plant-parasitic nematodes is when it is a seedling and its roots are growing within the top 20 cm of the soil. These results indicate that fluensulfone is mobile within the top 20 cm of all soils tested so its biological activity should not be hindered by soil type.

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Table 6.1 Soil properties for soils included in the fluensulfone adsorption and desorption study.

Sample Location	Soil Type	Taxonomy	Sand	Silt	Clay	OM	pH	CEC
			%	%	%	%	%	Meq/ 100g
Plains, GA	Greenville sandy clay loam	Fine, kaolinitic, thermic Rhodic Kandiudults	51	12	37	2	5.6	7.7
Tifton, GA	Tifton loamy sand	Fine, loamy, kaolinitic, thermic Plinthic Kandiudults	90	6	4	1	5.6	2.5
Athens, GA	Cecil sandy loam	Fine, kaolinitic, thermic Typic Kanhapludults	72	12	16	1.5	5.5	2.6
Citra, FL	Arredondo sand	Loamy, siliceous, semiactive, hyperthermic Grossarenic Paleudults	97	2	1	0.5	6.4	5.7
Kentucky	Sonora silt loam	Fine-loamy, mixed, semiactive, mesic Typic Paleudalfs	38	46	16	3.5	6.9	14
Texas	Tremona sand	Loamy, fine sand, thermic Aquic Arenic Paleustalfs	92	2	6	0.4	7.9	4.2
Colorado	Haxton sandy loam	Fine-loamy, mixed, superactive, mesic Pachic Argiustolls	60	26	14	1.4	8	26
Salinas, CA	Chualar sandy loam	Fine-loamy, mixed, superactive, thermic Typic Argixerolls	73	18	9	1.1	6.3	11.4
Yolo, CA	Yolo silt loam	Fine-silty, mixed, superactive, nonacid, thermic Mollic Xerofluvents	44	29	27	1.6	7.1	23.2

[z]Soil information was provided by University of Georgia Soil Testing Laboratory. Athens, GA and Waters Agricultural Labs. Camilla, GA.

Table 6.2 Sorption coefficient estimates for fluensulfone in soils included in the adsorption-desorption study[y].

Soil	OM %	K_F±SE[z]	1/n±SE	R^2	K_{FOC}
Greenville sandy clay loam	2.00	2.40±0.15	0.61±0.03	0.95	213
Tifton loamy sand	0.96	1.24±0.12	0.94±0.04	0.97	231
Cecil sandy loam	1.51	1.94±0.11	0.66±0.02	0.96	229
Arredondo sand	0.45	2.71±0.19	0.55±0.03	0.93	1075
Sonora silt loam	3.53	3.28±0.21	0.55±0.03	0.98	166
Tremona sand	0.41	1.26±0.09	0.74±0.03	0.95	548
Haxton sandy loam	1.42	1.50±0.16	0.780.05	0.96	188
Chualar sandy loam	1.06	1.69±0.09	0.71±0.05	0.97	285
Yolo silt loam	1.60	2.43±0.15	0.71±0.04	0.93	271

[y] Adsorption data was fitted to log form of Freundlich isotherms log C_S= log K_F + 1/n log Ce, where Cs (µmol/kg) is the amount of fluensulfone adsorbed at the equilibrium concentration Ce (µmol/L). K_F and 1/n are constants describing sorption capacity and sorption.
[z] Standard Error.

Table 6.3 K_D + standard error values for fluensulfone on different soils at five concentrations[z].

	Fluensulfone concentration (µMol/L)				
Soil type	1	5	10	20	40
Greenville sandy clay loam	2.29±0.30	0.60±0.36	0.49±0.01	0.47±0.01	0.41±0.02
Tifton loamy sand	0.73±0.14	0.45±0.02	0.57±0.06	0.59±0.04	0.54±0.03
Cecil sandy loam	1.49±0.14	0.53±0.04	0.47±0.04	0.43±0.04	0.34±0.02
Arredondo sand	3.03±0.30	0.66±0.03	0.38±0.05	0.45±0.02	0.41±0.01
Sonora silt loam	3.86±0.25	0.77±0.02	0.62±0.02	0.62±0.07	0.54±0.05
Tremona sand	0.88±0.04	0.36±0.04	0.31±0.02	0.35±0.01	0.30±0.01
Haxton sandy loam	1.08±0.05	0.41±0.04	0.43±0.03	0.43±0.01	0.46±0.02
Chualar sandy loam	1.19±0.06	0.50±0.01	0.39±0.03	0.43±0.02	0.37±0.02
Yolo silt loam	2.04±0.29	0.62±0.07	0.61±0.05	0.64±0.05	0.62±0.02

[z] $K_D = C_S * 10 / C_E * 20$ where Cs (µmol/kg) is the amount of fluensulfone adsorbed at the equilibrium concentration Ce (µmol/L) and 10 is the amount of treated soil in grams and 20 is the amount of $CaCl_2$ solution added to each sample in ml.

Table 6.4 Percentage of fluensulfone desorbed from soil after a 24-hour desorption process.

	Fluensulfone desorption rate[y]			
	5 µMol/L[z]	10 µMol/L	20 µMol/L	40 µMol/L
Soil type	%	%	%	%
Greenville sandy clay loam	22	34	41	49
Tifton loamy sand	26	53	48	53
Cecil sandy loam	32	46	48	52
Arredondo sand	9	27	25	26
Sonora silt loam	25	38	43	47
Tremona sand	31	47	44	54
Haxton sandy loam	58	81	81	70
Chualar sandy loam	32	55	50	60
Yolo silt loam	35	41	43	43

[y] Means represent the percentage of fluensulfone desorbed within 24 h desorption process compared to the total concentration added to each sample.
[z] Initial concentration at which fluensulfone was applied

Table 6.5 Pairwise correlations of soil parameters to fluensulfone soil adsorption (K_F) and desorption rate.

	Adsorption (K_F)		Desorption rate	
Parameters	Correlation	P-value	Correlation	P-value
pH	-0.1472	0.3917	0.3825	**0.0213**
CEC	0.1571	0.3602	0.4442	**0.0066**
OM	0.6664	**<.0001**	0.0126	0.9416
Sand	-0.5447	**0.0006**	-0.1572	0.3598
Silt	0.545	**0.0006**	0.2141	0.2099
Clay	0.3407	**0.042**	0.0269	0.8761

Figures

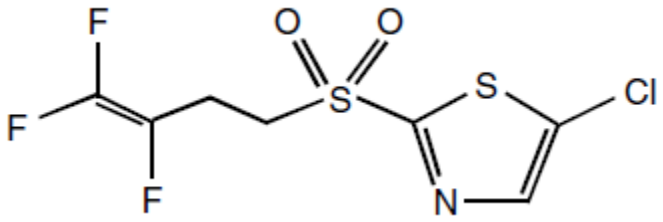

Figure 6.1 Chemical Structure of Fluensulfone

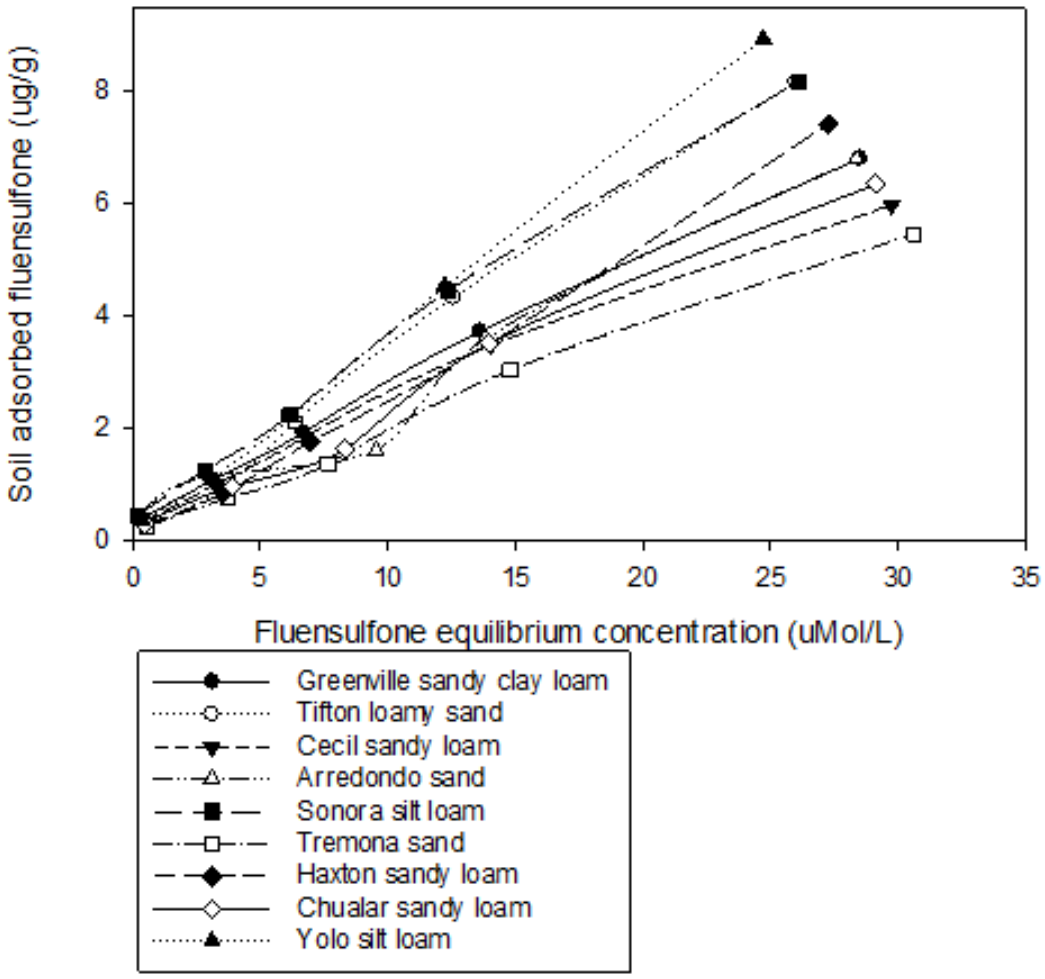

Figure 6.2 Adsorption isotherms of fluensulfone on 9 different soil types. Initial fluensulfone concentrations were 1, 5, 10, 20, and 40 µmol/L.

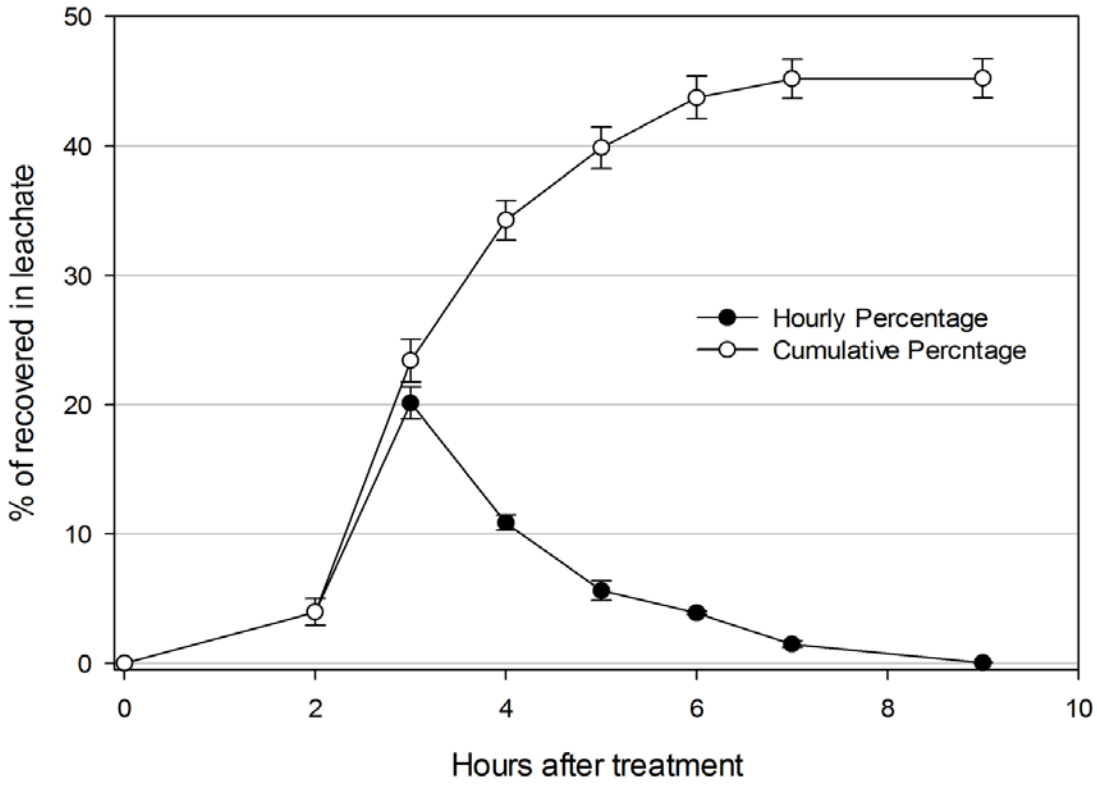

Figure 6.3 Mobility of fluensulfone on an Arredondo sand soil with 4000 ml irrigation.

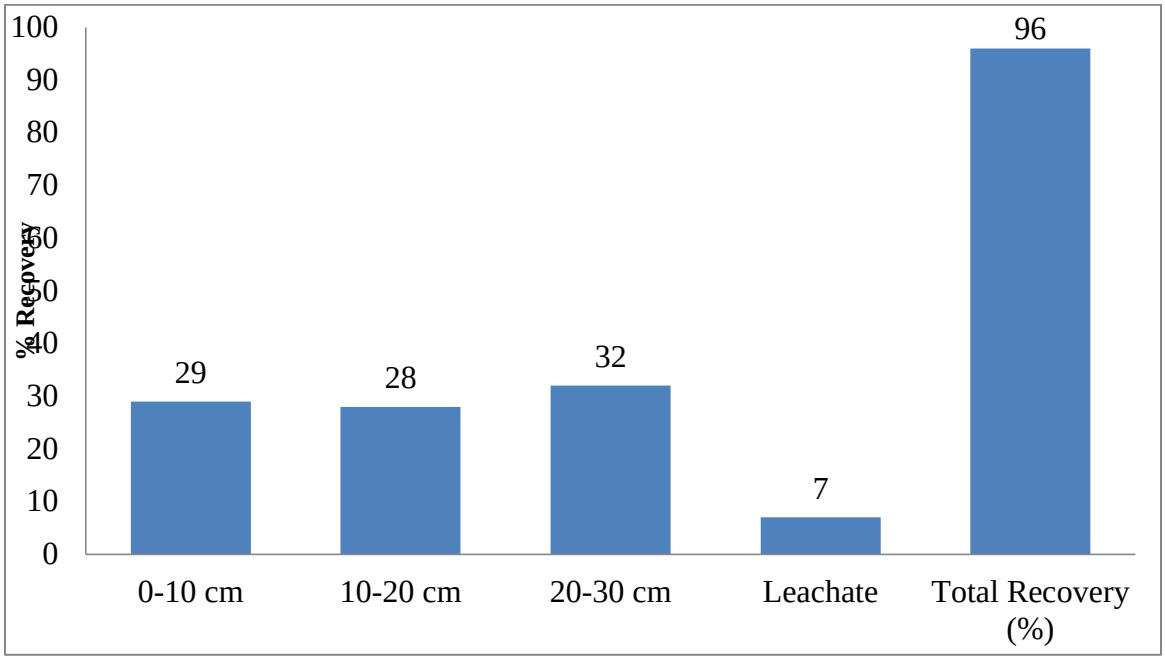

Figure 6.4 Mobility of fluensulfone on a Greenville sandy clay loam soil with 1000 ml irrigation.

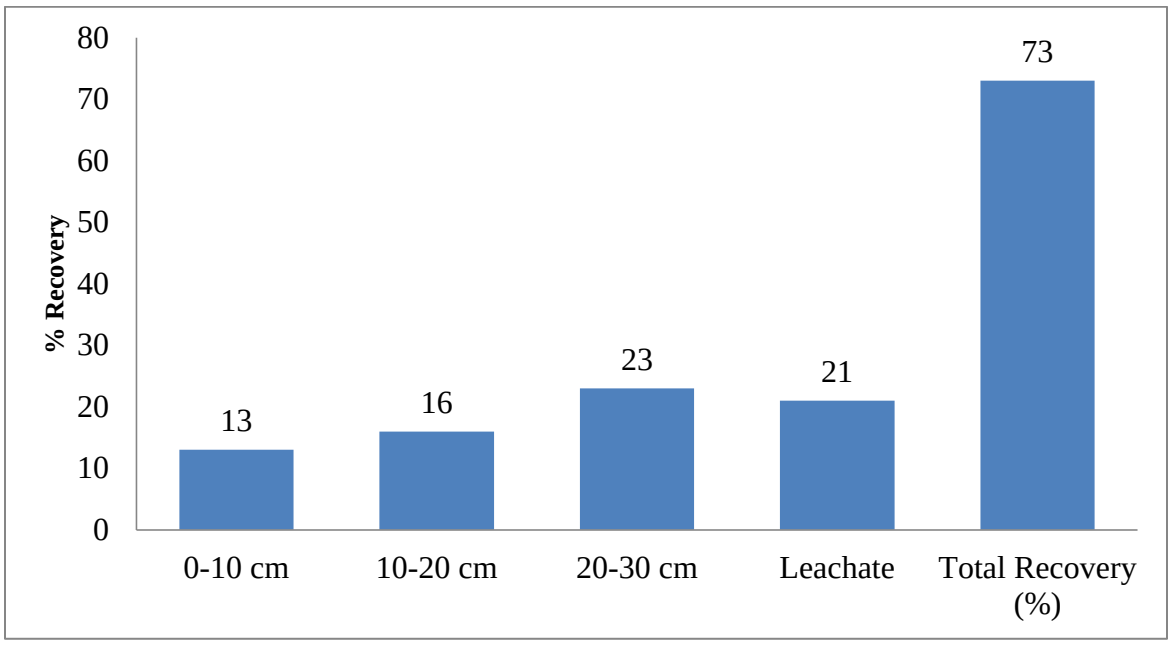

Figure 6.5 Mobility of fluensulfone on a Tifton loamy sand soil with 1000 ml irrigation.

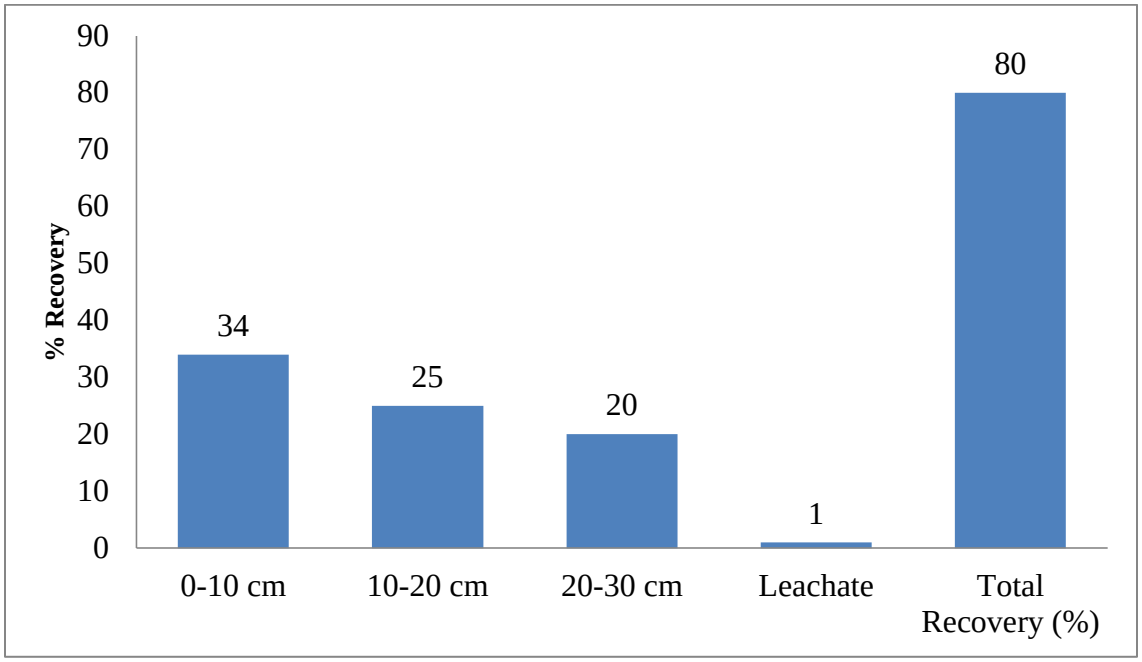

Figure 6.6 Mobility of fluensulfone on an Arredondo sand soil with 1000 ml irrigation.

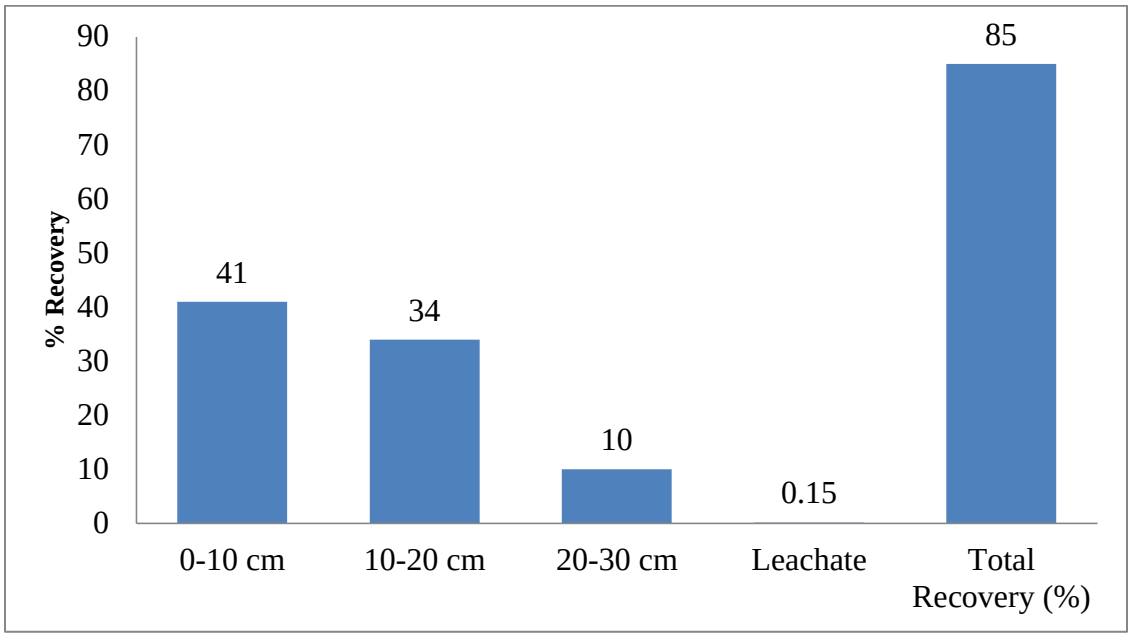

Figure 6.7 Mobility of fluensulfone on a Chualar sandy loam with 1000 ml irrigation.

CHAPTER 7

CONCLUSIONS

Root-knot nematode (RKN) (*Meloidogyne* spp.) is one of the most destructive genera of plant-parasitic nematodes that infects vegetable crops. Primary management of RKN is achieved through the use of nematicides, and in particular, fumigant nematicides. Methyl bromide has been used extensively for management of RKN in vegetables until its recent phase-out via the Montreal Protocol for being an ozone depleting substance. The loss of MeBr has sparked an increased interest into discovering MeBr alternatives. Other fumigant nematicides and biocides are available to growers, however; these products can be costly, difficult to apply, require fumigant management plans, and can pose significant environmental concerns. These challenges associated with fumigant applications realize the need for non-fumigant alternatives. Fluensulfone is a fluoroalkenyl non-fumigant nematicide that received EPA registration on cucurbits and fruiting vegetables in September 2014. There is limited published research on the efficacy of fluensulfone (Csinos *et al.*, 2010; Oka *et al.*, 2009), and there is no published data on field application methods of fluensulfone for RKN management. Field trials were conducted to evaluate the efficacy of fluensulfone when applied as a pre-plant incorporation or by different drip application methods. Both PPI applications and drip applications provided control of RKN on cucumber. Fluensulfone was applied PPI to first crop tomato and reduced nematode galling to the same effect as the industry standard fumigant1,3-

dichloropropene. In addition, a drip application on second crop cucumber following tomato resulted in a reduction in nematode gall ratings and an increase in cucumber yield.

The systemic activity of fluensulfone for control of RKN has been reported on pepper (Oka *et al*., 2012). Growth chamber studies were designed to evaluate crop sensitivity and the basipetal systemic activity of fluensulfone for control of RKN on cucumber, squash, tomato, and eggplant. When fluensulfone was applied only to the foliage of plants crop sensitivity was noted in all crops tested except squash. Systemic control of RKN was observed only in tomato. Oxamyl was used in these trials as the industry standard. Surprisingly, oxamyl demonstrated a reduction in the number of RKNs only in the roots of tomato. This data suggest that the systemic activity of both oxamyl and fluensulfone is crop dependent and future studies should be conducted on other crops to determine is foliar applications of fluensulfone can manage RKN damage.

The adsorption-desorption and mobility of fluensulfone in various soils was evaluated. Adsorption of fluensulfone was found to be positively correlated to soil organic matter content, silt content, and clay content and negatively correlated to sand content. Desorption of fluensulfone was found to be correlated to soil pH and soil cation exchange capacity (CEC). Fluensulfone adsorbed to a Sonora silt loam, Arredondo sand, Yolo silt loam, and a Greenville sandy loam more strongly than any other soil type tested. The mobility of fluensulfone was evaluated through four soils. Fluensulfone was less mobile through a Chualar sandy loam and an Arredondo sand than it was through a Tifton sandy loam and a Greenville sandy clay loam. It is surprising that fluensulfone adsorption was increased and mobility decreased in the Arredondo sand, considering that this soil type is >96% sand with little organic matter. Factors other than silt, clay, and

organic matter content probably contribute to the affinity of fluensulfone to bind to this soil. Aluminum oxide and iron oxide have been found to be efficient adsorbents of hydrophobic compounds (Pierce *et al*., 1971). However, the concentration of these compounds was not measured in the soil.

Many disease complexes have been associated with RKN and various soil-borne fungi (Back *et al.,* 2002). Growth chamber studies were conducted to evaluate the potential synergistic disease complex between *Pythium aphanidermatum* and *Meloidogyne incognita* on cucumber. When both pathogens occurred simultaneously in the soil an increase in seedling damping off was recorded compared to when either pathogen occurred by themselves. In addition, a reduction in plant vigor was noted when the two pathogens occurred together.

The registration of fluensulfone marks the first nematicide to receive EPA registration in over 20 years. Results from this work consistently demonstrated that fluensulfone can provide growers with an effective nematode management tool that can be applied via drip application or PPI. The ease of application, zero hour re-entry period, no buffer zone, and 'Caution' signal word are benefits that fluensulfone has over fumigant nematicides. The mobility of fluensulfone is dependent upon soil type and irrigation regiments should be imposed after application and prior to planting insure adequate bed coverage and to prevent any potential crop injury. In addition, future studies should be conducted to determine the efficacy of fungicides and nematicides for control of *Pythium* damping off in fields with high nematode densities.

Literature Cited

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