

Managing Weeds and Soilborne Pests with Fumigant and Non-Fumigant
Alternatives to Methyl Bromide

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Alternative

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ABSTRACT

Methyl bromide (MBr) was widely used as a soil fumigant to manage soilborne pests in plasticulture vegetable production; however, it has been banned by the United Nations Environment Programme. Alternatives to MBr must be implemented to sustain fresh market tomato productivity. Possible MBr alternatives include new fumigant compounds, improved plastic mulch, and grafting. Methyl iodide (MeI) and dimethyl disulfide (DMDS) were tested as fumigant alternatives to MBr for the control of yellow nutsedge and soilborne pathogens of tomato. Virtually impermeable film (VIF) and totally impermeable film (TIF) were tested for fumigant retention and yellow nutsedge control in tomato. Grafting onto resistant rootstocks was tested for bacterial wilt and nematode management in tomato. In the absence of a soil fumigant, TIF suppressed yellow nutsedge better than VIF. TIF increased fumigant retention compared to VIF at similar application rates. Reduced fumigant application rates could be used in combination with TIF while maintaining fumigant concentrations, weed control, and crop yields comparable to greater use rates with VIF. Shank applied DMDS rates could be lowered to 281 L/ha under TIF from 468 L/ha under VIF; shank applied MeI application rates could be reduced to 56 L/ha under TIF compared to 93 L/ha under VIF and drip applied DMDS could be reduced from 561 L/ha under VIF film to 374 L/ha under TIF. Grafting susceptible commercial tomato cultivars onto resistant tomato hybrid rootstocks increased yields and plant survival in bacterial wilt infested fields. 'Cheong Gang', 'BHN 998', and 'BHN 1054' were the best performing rootstocks for bacterial wilt resistance and tomato fruit yield in severely infested fields. Grafting increased tomato yield and decreased root galling from root-knot nematodes in an infested field. Hybrid

rootstock 'RST 106' resulted in the lowest root-knot nematode galling. In conclusion, TIF with reduced rates of DMDS or MeI is a viable MBr alternative for fresh market tomato production to retain effective doses of fumigant, manage yellow nutsedge and maintain yields. Grafting is an effective MBr alternative to manage bacterial wilt and root-knot nematode and maintain tomato yields.

I dedicate my dissertation to my future wife Camille Esmel.

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Introduction

Virginia ranked 3rd or 4th in the United States for harvested fresh tomato (*Solanum lycopersicum* L.) acreage (1821 to 1942 ha) and generated approximately 48 to 63 million dollars in production value per year between 2009 to 2011 (USDA-NASS, 2012). Fresh market tomato is the number one vegetable commodity in Virginia. Approximately 90% of the tomato acreage in the state is on the Eastern Shore (Wimer et al., 2009). The use of methyl bromide (MBr) has been instrumental in the management of pests in tomato production. MBr is a broad-spectrum biocide that is injected into the soil as a liquid, which subsequently vaporizes filling soil air space. For decades, tomato producers have relied on this fumigant for the management of soilborne diseases, nematodes, and weeds.

However, MBr was found to deplete the stratospheric ozone layer. MBr has been incrementally phased out by the Montreal Protocol on Substances that Deplete the Ozone Layer (UNEP, 2006a). During the phase out, use caps were developed for MBr, which declined annually. The use of MBr in field tomato production still continues under critical use exemptions (UNEP, 2006b). Under the Protocol (Decision IX/6) “a use of MBr should qualify as ‘critical’ only if the nominating Party determines that: (i) The specific use is critical because the lack of availability of MBr for that use would result in a significant market disruption; and (ii) there are no technically and economically feasible alternatives or substitutes available to the user that are acceptable from the standpoint of environment and public health and are suitable to the crops and circumstances of the nomination”. Critical use of MBr has been approved by the United States Environmental Protection Agency (US EPA) for pre-plant use in tomatoes grown in Virginia until 2013 (US EPA, 2012a). Use of methyl bromide in tomato should be limited to managing moderate to severe infestations of yellow (*Cyperus esculentus* L.) or purple (*Cyperus rotundus* L.) nutsedge, soilborne diseases, nematodes, and for research purposes. Effective methyl bromide alternatives must be found to sustain tomato production in Virginia and the United States.

Many producers have transitioned to alternative fumigants such as chloropicrin, 1,3-dichloropropene, metam sodium, metam potassium, and iodomethane. Most of these are older chemistries and were not previously used in favor of MBr due to issues such as lengthy plant-back periods and narrower pest management spectrum. In 2008, the EPA reregistered older pesticides (those registered before November 1984) under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) to meet current scientific and regulatory standards (US EPA 2008). Reregistration considered human health and ecological effects and resulted in actions to reduce risks. Product reregistration implemented reregistration eligibility decisions (REDs) that require risk mitigation measures be reflected on pesticide product labels. Reregistered soil fumigants include chloropicrin, dazomet, metam sodium/potassium, methyl bromide, methyl isothiocyanate, and 1,3-dichloropropene. Soil fumigant risk mitigation measures include, but are not limited to, reducing maximum application rates, requiring respiratory protection, and implementing buffer zones. According to the US EPA (2012d), “a buffer zone provides distance between the application site (i.e., edge of field) and bystanders, allowing airborne residues to disperse before reaching the bystanders”.

These mitigation measures have the potential to place extreme economic burden on producers causing current soil fumigation practices to be no longer economically feasible. These aspects have forced many tomato producers to seek alternative measures to manage soilborne

pests. Possible MBr alternatives include new fumigant compounds (including delivery methods of these fumigants), improved plastic mulch, and grafting.

This research focuses on MBr replacements that comply with current mitigation requirements for fumigants. Dimethyl disulfide (DMDS) and methyl iodide (MeI) will be evaluated as alternative fumigants to methyl bromide to control yellow nutsedge in fresh market tomato in Virginia. These fumigants will be tested using two application methods, the standard shank application and the novel application method through the drip irrigation system. Drip application of fumigants results in less fumigant handlers and thus lowers respiratory equipment costs. In conjunction with these fumigants, virtually impermeable film (VIF) and totally impermeable film (TIF) will be tested for fumigant retention for the possibility of decreasing application rates. Lastly, the potential of grafting tomato on resistant rootstocks for the management of bacterial wilt (*Ralstonia solanacearum* (Smith) Yabuuchi et al.) and root knot nematode (*Meloidogyne incognita* (Kofoid and White) Chitwood) will be evaluated. The goal of these experiments was to identify feasible alternatives to MBr that are compliant with current government regulations. Specific goals were to reduce application rates of shank applied DMDS and MeI and drip applied DMDS under TIF while maintaining fumigant concentrations, yellow nutsedge management and tomato yields. An additional aim was to manage bacterial wilt and root-knot nematodes with grafting onto resistant hybrid rootstocks in tomato while maintaining fruit yields and quality.

Chapter 1 Literature Review

Pests Managed by Methyl Bromide

Bacterial Wilt

Bacterial wilt (*Ralstonia solanacearum* (Smith) Yabuuchi et al.) was first described by Erwin F. Smith as the causal agent of a wilt disease in solanaceous plants (Smith, 1896). He was able to perform Koch's postulate on the disease to show that it was the pathogen responsible for bacterial wilt in tomato, eggplant, and potato. Important crop hosts include banana, eggplant, ginger, peanut, pepper, potato, tobacco, and tomato (Kelman et al., 1994). It has been reported on every continent and most islands in warm temperate and tropical regions of the world (Kelman et al., 1994). Bacterial wilt has been detected in river water when infected bitter-sweet nightshade (*Solanum dulcamara* L.) was present upstream during warm summer and fall months (Elphinstone et al., 1998). In addition, the pathogen can persist exceptionally well in the soil on the roots of asymptomatic plants, weeds, and plant debris (Elphinstone et al. 1998, Granada and Sequeira, 1983, Hayward, 1991). This disease is considered the most important phytopathogenic bacterial plant disease because of its extensive host range (over 200 species), worldwide distribution, and significant economic damage (Kelman, 1998). Furthermore, indirect losses due to fallowing of infested land results in a significant reduction of food production. The origins of the pathogen are unclear. It was present in Japan 200 years before the description of the causal agent. However, it has also been found in Solanaceous crops that were planted on newly cleared forest land in many tropical and subtropical areas (Kelman et al., 1994).

Bacterial wilt race 1 (biovar 1, phylotype II) is widely distributed in the southeastern United States and causes considerable economical losses under ideal conditions for disease incidence (Ji et al., 2007). Although most soilborne pathogens have been traditionally managed with soil fumigants, this strategy has been minimally effective against bacterial wilt (Chellemi et al., 1997; Enfinger et al., 1979). Crop rotation as a disease management strategy is effective but can be difficult because bacterial wilt can infect over 200 plant species (Hayward, 1994). Although resistance is available in tomato cultivars 'Hawaii 7996', 'Hawaii 7997', and 'Hawaii 7998' (Chellemi, 1994, Grimault et al., 1995; Grimault and Prior, 1993; Scott et al., 1989), these cultivars have not been widely accepted due to poor horticultural traits such as small fruit, a trait linked with bacterial wilt disease resistance (Opena et al., 1990, Wang et al., 1998b). On the Eastern Shore of Virginia, it is not uncommon for fresh market tomato growers to lose as much as 30% or greater crop stand before the first harvest due to bacterial wilt (Freeman et al., 2009a).

Yellow Nutsedge

Yellow nutsedge (*Cyperus esculentus* L.) is a member of the Cyperaceae (sedge) family. Holm et al. (1991) described yellow nutsedge as a light green perennial sedge with three-sided stems that can reach a maximum height of one meter. The plant has a basal bulb below ground that produces rhizomes. These rhizomes can produce either a secondary basal bulb or terminate in a single underground tuber. Upon sprouting these tubers produce rhizomes that produce more basal bulbs. Therefore the primary basal bulb creates a multitude of secondary, tertiary and higher order vegetative shoots capable of producing more rhizomes and tubers (Holm et al., 1991). The tubers are sweet, oily, and fleshy. Due to these characteristics the tubers are eaten by humans and in some areas yellow nutsedge tubers are cultivated for pig feed. Yellow nutsedge is one of the world's worst weeds infesting 21 crops in more than 30 countries (Holm et al., 1991).

According to Holm et al. (1991) *Cyperus esculentus* is found on every continent. Generally it is considered a warm region weed; however, it has been advancing steadily into temperate zones. In fact, yellow nutsedge can now be found in Alaska. This weed is especially troublesome in eastern and southern Africa and in North and Central America (Holm et al., 1991). This weed is generally found in low ground, moist fields, heavily irrigated crops, along river banks and roadsides, and in ditches. It grows well on all soil types and performs well at a pH range of 5 to 7. This weed does not tolerate shade. Yellow nutsedge has become a more prominent weed within the United States due to a shift in herbicide programs focused towards managing annual weeds, a decline in hand hoeing, and an increase in reduced tillage practices. Yellow nutsedge is found in every state and infests more than 1 million hectares in the eastern half of the United States (Holm et al., 1991).

The tubers of yellow nutsedge exhibit dormancy. This is a very important factor that relates to controlling yellow nutsedge with herbicides and tillage. Several experiments have shown higher tuber sprouting with overwintering and mechanical disturbance. Tumbleson and Kommedahl (1962) found 95 percent germination in spring harvested tubers compared with 12 percent germination in fall harvested tubers. In addition, they reported that storing tubers at 3°C promoted sprouting and increased shoot numbers per tuber. Taylorson (1967) found yellow nutsedge tubers to be the most dormant in late summer and fall. He also found that tuber sprouting was best in winter and spring. Thomas (1967) and Thomas and Henson (1968) confirmed that storing tubers at low temperatures (4°C) increased germination. It was also discovered that scraping tubers with sand paper increased germination by 45% and treating the tubers with hydrogen peroxide increased sprouting by 30%. In terms of dispersal, most tubers were located within the top 15 cm of the soil (Bell et al., 1962).

Holm et al. (1991) reported *C. esculentus* to be a serious or principle weed in many crops worldwide. In the United States it is one of the most important perennial weeds and can be found in every state. Some other countries that have serious yellow nutsedge problems include Peru, South Africa, Swaziland, Angola, Tanzania, Mozambique, Zimbabwe, Canada, Kenya, Australia, Costa Rica, Taiwan, Chile, India, Portugal. Crops that frequently become infested with yellow nutsedge include sugarcane, corn, soybeans, potatoes, coffee, cereals, peanuts, sugar beets, pineapples, cotton, rice, tea, citrus, tobacco, and vegetables.

The primary weed controlled by methyl bromide in tomato production on the Eastern Shore of Virginia is yellow nutsedge. In the Southern United States, yellow nutsedge is among the most common and troublesome weeds in fruiting vegetables in Alabama, Georgia, Kentucky, North Carolina and South Carolina (Webster, 2006). Although purple nutsedge (*Cyperus rotundus* L.) is a devastating weed of tomatoes in many areas, it does not tolerate the temperate climate of Virginia. Yellow nutsedge is not completely controlled by plastic mulch because the plant has sharp leaf tips that readily puncture and emerge through the plastic. Black mulch does suppress yellow nutsedge spread in terms of shoot production and lateral expansion compared to a non-mulched control. A single yellow nutsedge tuber in black LDPE mulch produced 62 shoots by 24 weeks after planting compared to 208 shoots produced in the non-mulched control during the same time period (Webster, 2005a). In a greenhouse study, a pre-sprouted nutsedge tuber covered by black polyethylene produced 6 shoots above ground, 73 shoots below ground, and 179 tubers after 16 weeks (Webster, 2005b).

Relatively low infestations of yellow nutsedge can result in decreased tomato yields. A greenhouse study in Florida showed that a single pre-sprouted yellow nutsedge tuber planted with a tomato transplant in a 15 cm diameter container (equivalent density of 57 plants/m²)

reduced tomato dry weight by 34% compared to a tomato without nutsedge competition (Morales-Payan et al., 2003). In the same study it was found that yellow nutsedge competes extensively both above ground and below ground for resources. Tomato dry weight was reduced by 19% by either above ground or below ground competition alone. In a field study, Stall and Morales-Payan (2003) found that season long interference of 25 yellow nutsedge plants/m² resulted in a 10% marketable yield loss of tomato. In addition, they found the critical weed free period for yellow nutsedge in tomato to be 2 – 10 weeks after transplanting to avoid tomato yield losses above 5%.

Other studies have shown season long interference of yellow nutsedge resulted in yield loss in bell pepper (Motis et al., 2003; Santos et al., 2007a), watermelon (Buker et al., 2003), and cucumber (Johnson and Mullinix, 1999). Santos et al. (1997) found that tomato was a better competitor than yellow nutsedge, especially for light. Effectively managing yellow nutsedge with fumigation would allow tomato to form a canopy and out-compete yellow nutsedge. Purple nutsedge interference resulted in up to 44% fruit yield loss for tomato and 32% for bell pepper (Morales-Payan et al., 1997).

In addition to reducing crop yields the weed is considered troublesome due to lowered quality of farm produce. It has been reported that every potato tuber was found to have a rhizome penetrating it, in severely infested potato fields (Bell et al. 1962). Infestations increase production costs due to increased cultivation, hand weeding, herbicide applications, and fumigation treatments.

Nematodes

Nematodes are tiny round worms that comprise a large phylum of animals (phylum Nematoda) that includes free living species, animal parasites, and plant parasites (Williamson and Hussey, 1996). Plant parasitic nematodes are obligate parasites. Sedentary endoparasitic nematodes belonging to the Heteroderidae family cause the most economic damage worldwide (Williamson and Hussey, 1996). This family is divided into two groups: the cyst (genera *Heterodera* and *Globodera*) and the root-knot nematodes (genus *Meloidogyne*). Root-knot nematodes form characteristic galls on host plants and infect thousands of plant species resulting in major yield loss in many crops. Symptoms of nematode infected plants include stunted growth, wilting, and susceptibility to secondary pathogens.

In California, root-knot nematode (*Meloidogyne* spp.) was the primary problematic nematode species, which resulted in an estimated 2% yield loss in fresh market tomato and 10-20% yield loss in processed tomato in 1994 (Koenning et al., 1999). In California, approximately 80% of the fresh market tomato crop and 25 % of the processing tomato crop received nematicides (primarily soil fumigation) to manage root-knot nematode (Koenning et al., 1999). In Virginia, the plant parasitic nematode genera *Meloidogyne* had the greatest impact on solanaceous vegetable production and resulted in an estimated 1-5% crop loss in 1994 (Koenning et al., 1999).

Alternative Fumigants to Methyl Bromide

Dimethyl Disulfide

The USDA has identified several research needs and priorities for methyl bromide alternatives in solanaceous crops (USDA, 1993). Short term goals include, but are not limited to, extensively evaluating alternate chemicals by themselves or in combinations and re-evaluating

existing germplasm for resistance/tolerance to major soilborne plant diseases. A long-term goal identified by the USDA workshop was to develop integrated pest management systems, including chemical and non-chemical approaches for management of soil pests. The USDA lists broad spectrum fumigants and plastic mulch as existing and potential alternatives to methyl bromide. In addition, the US EPA lists dimethyl disulfide (DMDS) as a methyl bromide alternative for tomato (US EPA, 2012c). DMDS fumigant has recently been registered by the EPA and is marketed under the trade name Paladin®. DMDS is registered for pre-plant use in tomato, pepper, eggplant, cucurbit crops, and strawberries grown in plasticulture for the management of nematodes, weeds, and soilborne plant pathogens.

DMDS is predicted to have a half-life of approximately several hours and is considered to have no ozone depleting potential (Robinson et al., 2006). It is a natural compound that is part of the global sulfur cycle. DMDS is produced by living organisms in wetlands and oceans as well as members of the plant families *Alliaceae* and *Brassicaceae* (McKown, 2010). It is believed to be produced by plants as a defensive response to damage. Naturally occurring DMDS is found in a variety of crops and foods in measurable amounts (McKown, 2008).

Experiments have been conducted to test the efficacy of DMDS to control yellow nutsedge in tomato and cantaloupe in Florida (Olson and Rich, 2007). In tomato, DMDS formulated with chloropicrin (Pic) (DMDS:Pic 79:21 w/w) was applied at two rates (549 and 823 kg/ha) under three types of plastic mulch (low density polyethylene (LDPE), virtually impermeable film (VIF), and metalized). These treatments were compared with methyl bromide fumigation at two rates (196 and 392 kg/ha) and non-treated checks under the three types of plastic mulches for the control of yellow nutsedge in tomato. All DMDS treatments controlled nutsedge (≤ 30 plants/9 m of row) better than the non-treated checks (147.3 -309.5 plants/9 m of row) and were similar to methyl bromide (≤ 3.5 plants/9 m of row). Furthermore, there was no difference in total tomato yields between DMDS (5380- 6597 11.4 kg boxes/ha) and methyl bromide (4604 -5733 11.4 kg boxes/ha) treatments. DMDS and MBr treatments yielded greater than the non-treated VIF (1566 11.4 kg boxes/ha). In cantaloupe, pure (100%) DMDS was applied at two rates (448 and 646 kg/ha) under LDPE and VIF. These treatments were compared with methyl bromide fumigation, 1,3-dichloropropene, and a non-treated check. DMDS (168.5 plants/9 m of row) and methyl bromide (0 plants/9 m of row), both applied under VIF, provided similar control of yellow nutsedge. In addition, DMDS applied under VIF (168.5 plants/9 m of row) provided better yellow nutsedge control than 1,3-Dichloropropene (798 plants/9 m of row). There were no differences in cantaloupe yields between methyl bromide (48.6 to 49.7 kg/ha) and DMDS (38.9 to 44.9 kg/ha). However, cantaloupe yields were higher when treated with methyl bromide (48.6 to 49.7 kg/ha) compared to the non-treated check (34.3 kg/ha).

Many growers in Georgia have adopted a 3-way system using 1,3-Dichloropropene, chloropicrin, and metam sodium as a methyl bromide alternative (Culpepper et al., 2008). Experiments were conducted to compare the 3-way applied under LDPE mulch with three formulations of DMDS (DMDS, DMDS:Pic 90:10, and DMDS:Pic 79:21 w/w), applied at four rates (281, 374, 468, and 561 L/ha) under VIF film. An additional treatment of DMDS was applied under LDPE mulch. All DMDS treatments ($\geq 90\%$ control) controlled purple nutsedge (*Cyperus rotundus* L.) better than the non-treated check (51% control). DMDS under VIF ($\geq 96\%$ control) and the 3-way system (100% control) provided excellent nutsedge control. The 3-way system (98% control) and all the DMDS treatments ($\geq 33\%$ control) provided superior large crabgrass (*Digitaria sanguinalis* (L.) Scop) control compared to the non-treated check (4% control). DMDS:Pic 79:21 at 468 L/ha (86% control) and 60 GPA (92% control) controlled

crabgrass similarly to the 3-way. Welker et al. (2006) found no significant differences in total weed control between MBr:Pic (196 kg/ha under VIF and 392 kg/ha under LDPE) and DMDS at 701, 589, and 468 L/ha under VIF. There were no differences in tomato marketable fruit yields between MBr and DMDS treatments under VIF.

DMDS has been tested as a methyl bromide alternative in strawberries. Othman et al. (2010) found no significant differences in mean strawberry yields between DMDS (392 kg/ha) tank mixed with Pic (112 kg/ha) or sequential application of Pic (112 kg/ha) followed by DMDS (392 and 493 kg/ha) and MBr:Pic 57:43 (w/w). The study suggested that DMDS:Pic can control soil pathogens such as *Pythium* spp. and weeds such as annual grasses and *Amaranthus* spp. Welker et al. (2007) found no significant differences in weed incidence between DMDS:Pic (79:21 w/w) under VIF and MBr (67:33 w/w) under LDPE treatments. MBr and DMDS at two rates (355 and 589 L/ha) had less weed incidence than the non-treated controls (non-fumigated VIF and LDPE treatments). In addition, strawberry marketable yields were similar between MBr and DMDS treatments. Lopez-Aranda et al. (2009) found that DMDS:Pic (50:50 w/w at 500 kg/ha) consistently improved early and total marketable high tunnel strawberry yields in two locations in Spain. The higher yields and increased plant canopy diameters were attributed to soilborne fungi and nematode control. Garcia-Mendez et al. (2008) reported that DMDS:Pic (50:50 and 73:27 w/w) under VIF decreased weed density and fresh weed weight compared to the non-fumigated control and provided similar weed control as MBr:Pic (50:50 w/w) under PE in strawberry runner production. In the same experiment, DMDS:Pic provided similar total marketable runner plant production as MBr:Pic in most cases. Pure DMDS (100% DMDS) under PE did not control fungal pathogens in Spanish strawberry nurseries (De Cal et al., 2004).

Methyl Iodide

Methyl iodide (MeI), also referred to as iodomethane, is an alternative to MBr for pre-plant soil fumigation (Duniway, 2002). Title 5, section 602 of the Clean Air act orders the U.S. EPA to list any substance with an “Ozone Depletion Potential” (ODP) of 0.2 or greater as a Class 1 ozone depleter. The ODP of MeI is likely less than 0.016, which is much lower than the level of Class 1 ozone depleters (Ohr et al., 1996). MeI is produced by marine algae and is uniformly distributed in the ocean. The gas is a general biocide like MBr. MeI is the most effective fumigant compared to other alkyl iodides. Ohr et al. (1996) describe MeI as: being a better methylating agent than MBr and equal or better at suppressing certain soilborne pathogens and weeds than MBr at equivalent molar rates. MeI is an ozone safe alternative to MBr due to rapid degradation by UV light and a logical candidate as a single chemical replacement for MBr (Ohr et al., 1996). MeI does not persist long in the atmosphere having a residence time of only 4 to 8 days compared with 2 years for MBr. Numerous studies report similar findings. Gan and Yates (1996) reported that MeI movement is slower in the soil profile and that soil surface volatilization will be less on the same time scale. However, MeI degradation is slower than MBr in soil, therefore, under similar application and soil conditions, the overall MeI loss from volatilization and the risk of entering the ground water would likely be greater than MBr. However, in water MeI may dissipate rapidly if the water is exposed to air and sunlight (approximately 1 day half life) (Gan and Yates, 1996).

Experiments in California indicated that MBr or MeI used in combination with clear or black polyethylene mulch was effective at controlling purple nutsedge. There was not a significant difference in purple nutsedge control between the two fumigants. In addition, MeI

was equally or more effective than MBr at controlling purple nutsedge germination from different depths in the soil (Ohr et al., 1996).

In a dose response experiment conducted in a fumigation chamber on yellow nutsedge, the EC₅₀ (effective concentration to provide 50% control) for MeI applied alone was 2.6 times less than for MBr (Hutchinson et al., 2003). This indicates that 2.6 times less chemical was needed to provide similar levels of control, thus MeI is more efficacious at controlling yellow nutsedge compared to MBr. Combining MeI with 17% Pic resulted in a synergistic response. The relative potency of MeI increased 1.7 times when 17% Pic was added. There was no significant difference between the EC₅₀ values of MBr and MeI when both fumigants were combined with Pic (Hutchinson et al., 2003).

A separate laboratory bioassay experiment also indicated that MeI was more potent at controlling weeds than MBr. Zang et al. (1997) found that the dose of MeI needed to control redroot pigweed (*Amaranthus retroflexus* L.) was similar to MBr and that MeI was more effective at controlling lambsquarters (*Chenopodium album* L.), purple nutsedge (*Cyperus rotundus* L.), yellow nutsedge (*Cyperus esculentus* L.), wild mustard (*Brassica kaber* (D.C.) L.C. wheeler), Italian ryegrass (*Lolium multiflorum* Lam.), velvetleaf (*Abutilon theophrasti* Medik.), and common purslane (*Portulaca oleracea* L.). Yellow nutsedge was more sensitive than purple nutsedge and less sensitive than redroot pigweed to MeI fumigation. Yellow nutsedge, Italian ryegrass, velvetleaf, lambsquarter, common purslane, and wild mustard were similar in sensitivity to MeI.

Hutchinson et al. (2000) found that MeI was 2.7 times more efficacious than MBr when averaged over nine fungal species. Furthermore, MeI combined with Pic was 2.8 times more efficacious against *Fusarium oxysporum* (Schlechtend:Fr) than MeI applied alone. MeI was more effective than MBr against the following plant parasitic nematodes (*Meloidogyne incognita*, *Heterodera schachtii*, and *Tylenchulus semipenetrans*) (Becker et al., 1998; Hutchinson et al., 1999a, Hutchinson et al., 1999b) and the plant pathogenic fungus (*Rhizoctonia solani*) (Becker et al., 1998). Noling et al. (2009) reported that MeI provided good to excellent management of nematodes, diseases, and weeds compared to MBr and other MBr alternative fumigants.

MeI is more costly than other soil fumigants, therefore reduced application rates would benefit growers (Gilreath and Santos, 2011). Olson and Kreger (2007) conducted field experiments in north Florida to test the efficacy of MeI as a soil fumigant for tomato production. The experiment was conducted over two years (2006 and 2007). The first year, various rates of MeI and MBr were applied under metalized and black low density polyethylene (LDPE) mulch types. The second year various rates of MBr and MeI were applied under VIF and metalized mulch types. MeI was formulated with 50% Pic and the MBr was formulated with 33% Pic in both experiments. In 2006, reduced MeI rates (168 -252kg/ha) under metalized mulch provided similar control of yellow nutsedge compared to MBr at 392 kg/ha under LDPE. Under LDPE a rate of 280 kg/ha of MeI was needed to equal MBr control. The greatest total marketable and extra large tomato fruit yield was obtained with MeI applied at a rate of 252 kg/ha under LDPE. This was significantly greater than the non-treated check under LDPE and MeI applied at 168 and 224 kg/ha under metalized mulch. In 2007, all fumigation treatments controlled yellow nutsedge better than the non-treated checks. In addition, the non-treated metalized mulch reduced yellow nutsedge counts compared to the non-treated VIF mulch. MBr applied at a rate of 392 kg/ha under metalized mulch yielded the most extra large fruit, which was significantly greater than the non-treated checks under both VIF and metalized mulch. MeI at a rate of 196

kg/ha under VIF resulted in the highest total yield, which was significantly higher than the non-treated checks. Gilreath and Santos (2011) found that MeI:Pic (98:2 and 50:50 w/w) applied at rates of 140 and 224 kg/ha, respectively, under HDPE provided the greatest marketable tomato fruit yields and provided the greatest control of purple and yellow nutsedge. These results were similar to a MBr:Pic treatment (67:33 (w/w) at a rate of 392 kg/ha).

Drip Applied Fumigants

Chemigation is the process of applying chemicals to the soil or plant through irrigation water (Ajwa et al., 2002). Applying chemicals through irrigation may provide more uniform distribution without disturbing or compacting the soil. This system has been proposed to deliver soil fumigants in order to reduce handler exposure, because fewer workers are need for application. Therefore, drip applying fumigants can save growers money by reducing worker respirator equipment. Drip fumigation under high density polyethylene (HDPE) film resulted in more uniform distribution of 1,3-dichloropropene (1,3-D) in soil compared to a shank injected application (Ajwa et al., 2002). In addition, the process requires little extra equipment and energy. Emulsifiable concentrate (EC) fumigant formulations can be applied through the same irrigation systems that are subsequently used to water the crop. Fumigant application through drip irrigation can be economical, and is likely to reduce emissions, worker exposure, and application rates compared to conventional shank application (Ajwa et al., 2002). Ou et al. (2005) suggest that drip fumigants should be more effective than shank delivered fumigants because the chemical will be dissolved in the water phase and directly contact soil microorganisms which are coated in a layer of water. Effective management of citrus nematode (*Tylenchulus semipenetrans* Cobb) was achieved with a reduced application rate (47 kg AI/ha) of 1,3-D drip applied and a higher rate (112 kg AI/ha) of 1,3-D shank applied, which confirms the potential to lower application rates using drip applications (Wang and Yates, 1999).

The effect of formulation (Telone EC (for drip application) vs. Telone II (for shank application)) on fumigant (1,3-D) fate may be insignificant in transformation rate and phase partitioning in water and soil (Kim et al., 2003). Soil sorption of 1,3-D isomers was not affected by formulation. Both application methods resulted in similar cumulative emissions for both isomers (45% for (*E*)-1,3-D and approximately 50% for (*Z*)-1,3-D) (Kim et al., 2003). Emissions in non-tarped soil columns were more rapid and produced greater maximum instantaneous flux in a shallow subsurface (10 cm) drip application of Telone EC compared to a deeper (30 cm) shank injection of Telone II. Kim et al. (2003) suggest that to reduce emission in drip application of fumigants, the fumigant must be applied at deeper depths to prevent rapid volatilization if the amount of water applied does not sufficiently restrict vapor diffusion.

Polyethylene is compatible with most fumigants, but polyvinyl chloride can only be exposed to high fumigant concentrations for short durations (Ajwa et al., 2002). When applying fumigants through the irrigation system it is important to check for uniform pressure (with pressure regulator) and leaks. If leaks or ponding occur, the application should be immediately terminated. The irrigation system should be flushed with water prior to application to remove residual fumigant.

The amount of irrigation applied in drip fumigant application is important for fumigant distribution and pest efficacy. In sandy loam soil, a minimum of 40 mm (40 L/m²) of water delivered through two drip tapes was needed to horizontally distribute the fumigant (30 cm) to the edge of the raised bed (76 cm bed) (Ajwa et al., 2002). Ajwa and Trout (2004), also found a large amount of irrigation water (50 L/m) may be needed to provide good lateral distribution of fumigants in sandy loam or loamy soils. Greater fumigant concentrations were found over a

longer period of time with larger irrigation amounts, suggesting that water reduces volatilization losses by increasing fumigant levels in the water phase and reducing the total air space in soil for fumigant diffusion.

The effects of number of drip tubes on drip applied fumigants have been tested. Drip applied emulsifiable 1,3-D (60.8% w/w 1,3-D and 33.3% w/w Pic) concentrations were greater in the center of the bed with a single tube application, while concentrations were greater midway between the center and at the bed shoulder with a double tape application (Desaeger et al., 2004). The width of the wetting pattern when dye was injected into the drip tape increased significantly (two to threefold on average) when two drip tapes were used compared to one. In addition, the width of the nutsedge control pattern was improved (13% on emitters and 17% between emitters on average) when using two drip tapes instead of one (Desaeger et al., 2004). The width of nutsedge control was increased further with two drip tapes with lower application rates (18% wider nutsedge control) compared to higher application rates (12% wider nutsedge control). A double drip tape configuration was more effective than higher irrigation volumes at improving water movement, uniform fumigation pattern, and nutsedge control (Desaeger et al., 2004).

Plastic Mulch

Plasticulture is defined as “a system of growing vegetable crops where significant benefit is derived from using products derived from polyethylene (plastic) polymers” (Lamont, 2005). Typical plasticulture fresh market tomato production utilizes raised beds covered with plastic mulch, drip irrigation, delivery of fertilizer and crop protectants through the drip irrigation (fertigation/chemigation), and pre-plant soil fumigation under the plastic mulch. Benefits of plastic mulch include earlier crop production (7-21 days earlier), higher yields per unit area (2-3x higher), more efficient water and fertilizer use, reduced fertilizer leaching, reduced soil erosion (wind and water), reduced soil compaction and root pruning, the ability to double crop, cleaner/higher quality produce and a reduction in disease, insect pests, and weeds (Lament, 1993).

Earlier yields are attributed to optimum soil temperature, which promotes more rapid development of crops and higher yields. Water efficiency is increased because plastic mulch has a high degree of impermeability to water vapor, therefore soil evaporative loss is decreased. Plastic mulches reduce light penetration into the soil which prevents weed growth. The most notable exception is nutsedge species which have nut-like tubers that provide enough energy for the sprouted plants to puncture the mulch and emerge (Lament, 1993). Excess water runs off the impervious mulch, which reduces fertilizer leaching (increase fertilizer efficiency) and soil erosion. Soil under the mulch remains loose and well aerated because machinery and walking on the beds surface is avoided. Harvested fruit and vegetables from a mulched crop are cleaner and have less disease because soil is not splashed onto the plants or fruits. Cultivation is eliminated which eliminates root pruning. Multiple crops can be grown and harvested while utilizing the mulch and drip irrigation from the original crop.

Vegetable crops have been produced using plastic mulch since the early 1960s (Lamont, 2005). Plastic mulch is typically made from either low or high density polyethylene (LDPE and HDPE), which are 0.01 to 0.03 mm thick and 122 to 152 cm wide (Lamont, 2005). HDPE reduces mulch weight and cost, while increasing strength compared to LDPE of the same thickness. Mulch can be either smooth or embossed with a diamond shaped pattern. The pattern allows expansion and contraction which avoids loosening of the mulch from the raised bed.

Raised beds are usually 10 to 30 cm high, 61-91 cm wide, and have a generally have a declining slope of 3.18 cm from the center to the shoulder to drain precipitation.

Virtually Impermeable Film (VIF) and Totally Impermeable Film (TIF)

Effect of VIF and TIF on Fumigant Emissions and Retention

Dosage (D) is the product of pesticide concentration (C) and the time (T) of exposure to the target organism (Lembright, 1990). Efficacy of a fumigant is determined by the dosage of that fumigant for a particular pest (Munnecke and Van Gundy, 1979). Several papers make reference to C x T values required to kill certain pathogens with a specific fumigant (Gamliel et al., 1998a; Minuto et al., 1999). Less permeable mulches may retain fumigants longer at a greater concentration. Adequate fumigant concentration within the soil may be achieved with lower fumigant application rates. Benefits of mulches with increased fumigant retention are a reduction in the amount of fumigant needed for effective pest management, lower emissions, and a decreased buffer zone requirement.

The most advanced high retention films are virtually impermeable film (VIF) and totally impermeable film (TIF). LDPE is the most permeable mulch to fumigants, followed by HDPE, and VIF (Noling 2002). Films manufactured by coextrusion containing multilayers with barrier polymers, such as ethyl vinyl alcohol (EVOH) or polyamide (nylon) are significantly less permeable to fumigants (Gamliel et al., 1998a; Gamliel et al., 1998b; Gamliel et al., 1997; Wang et al., 1998a; Yates et al., 2002; Ou et al., 2007; Santos et al., 2007b; Wang et al., 1997; Chellemi et al., 2011; Gao et al., 2011a; Gao et al., 2011b; Qin et al., 2011; Fennimore and Ajwa, 2011) than LDPE and HDPE. These less permeable films were all classified as VIF in the literature regardless of type of barrier material (Yates et al., 2002; Yates et al., 1998; Gilreath et al., 2005a; Gilreath et al., 2005b; Minuto et al., 1999; Samtani et al., 2010; Hamill et al., 2008; Austerweil et al., 2006; Noling et al., 2009; Santos et al., 2007b; Santos et al., 2006a; Santos 2006b; Ou et al., 2007; Wang et al., 1998a, Chellemi et al., 2011).

Films containing an EVOH barrier layer have subsequently been referred to as totally impermeable film (TIF) (Chow 2008; Fennimore and Ajwa, 2011; Qin et al., 2011; Gao et al., 2011b; Villahoz et al., 2008). Characteristics of TIF include good film handling properties and extremely low fumigant vapor permeation (Chow, 2009, Qin et al., 2011). Due to the lowered fumigant emissions provided by TIF, the US EPA has amended the re-registration eligibility decisions (REDs) to approve a 60% buffer zone reduction credit for MBr:Pic when applied under certain types of TIF (US EPA, 2009). A potential drawback of TIF is that the plant back period (period after fumigation until planting, measured in days, that is required by the label to prevent phytotoxicity of the crop) may be increased for certain fumigants.

In order to determine how well a film retains a fumigant, its permeability must be determined. In the past, film permeability was quantified as the amount of fumigant (grams) passing through the mulch cover per unit time (hr) per unit surface area (m^2) (Noling et al., 2009). Gamliel et al. (1997) considered films to be gas impermeable if they had a MBr permeability rate of less than $1 \text{ g/m}^2/\text{h}$ at a temperature of $50 \text{ }^\circ\text{C}$. The French Ministry of Agriculture standards specify that films classified as VIF could not exceed a permeability level of $0.2 \text{ g/m}^2/\text{hr}$ for MBr (Noling et al., 2009). The new standard measurement of fumigant permeability through films is the mass transfer coefficient. Unlike other measures of permeability the mass transfer coefficient is a property of the film-chemical combination and independent of the concentration gradient across the film (Papiernik et al., 2001, Papiernik et al., 2002). This method provides a sensitive and reproducible gauge of film permeability. New

classifications for high barrier, low permeability films are TIF, VIF, and semi-impermeable (SIF), which include metalized films (Noling et al., 2009). The US EPA has recently categorized and quantified the permeability of various agricultural films on the market to MBr and other fumigants under laboratory conditions at 25°C, at elevated temperature (40°C) and at an elevated relative humidity (at 25°C) (Qian et al., 2010). The Paladin (DMDS) label requires using only certain types of VIF and metalized mulches during application. DMDS has a strong sulfur odor (garlic-like smell) that can be perceived at very low concentrations. Therefore, it is important to retain applied DMDS fumigant with highly retentive films, especially near populated areas.

Chellemi et al. (2010) reported that in Georgia cumulative emissions of DMDS under VIF was 37.5% and under LDPE it was 53.9 and 95.4% at different sites. Under LDPE the largest DMDS emission spike was observed between 12 to 48 h after application, but was at detectable levels even 10 days after application. However, a large flux in DMDS emissions was prevented by VIF. Ashworth et al. (2011) found that the use of VIF dramatically reduced MeI emissions (0.04% of total applied) compared to the bare soil control (83% of total applied). Cutting the VIF after two weeks led to an immediate spike in MeI emissions, however, the total emissions remained low (6% of total applied). Based on these results VIF lowers MeI emissions and may increase soil gas concentrations, improve pest management efficacy, and likely allow for reduced application rates.

Virtually impermeable films can further reduce fumigant emissions following drip fumigant applications. Ajwa et al. (2002) found that an EC formulation of 1,3-D:Pic (65:35 w/w) resulted in greater fumigant concentrations under VIF compared to HDPE. Ou et al. (2005) found VIF retained shank or drip applied 1,3-D and Pic better than PE. Cumulative emissions of drip applied methyl isothiocyanate (MITC) and 1,3-D were decreased by $\geq 80\%$ using VIF compared to HDPE (Papiernik et al., 2004). In a laboratory study, stimulated drip applied emissions were lower for 1,3-D and Pic under VIF compared to HDPE (Ashworth et al., 2010). Under the VIF, greater concentrations and wider distribution of fumigants were observed. Therefore, reduced fumigant rates may be equally or more effective under VIF compared to standard rates under HDPE, while improving fumigant distribution and lowering emissions.

TIF has been compared to standard polyethylene films for fumigant retention, emission, and distribution in the soil. 1,3-D:Pic 61:35 (w/w) concentrations were 46% to 54 % greater under TIF than HDPE (Fennimore and Ajwa, 2011). Peak emissions flux of 1,3-D plus Pic 50:50 (w/w) was greatly reduced in a field covered in TIF compared to a field covered in standard PE. Total emission loss through TIF film was 2% for 1,3-D and $<1\%$ for Pic during a six day period, while emission loss through standard PE film was 43% for 1,3-D and 12% for Pic (Qin et al., 2011). There was a greater emission surge of 1,3-D at film cutting in the TIF field compared to the standard PE field, however Pic emissions were low in both fields. TIF allowed for greater 1,3-D plus Pic concentrations and a more uniform distribution in the soil, compared to PE, therefore growers may be able to manage pests with lower fumigant rates when using TIF (Qin et al, 2011). However, the surge of 1,3-D after cutting TIF could pose a risk to workers and bystanders. Reducing total emissions will benefit the environment and bystanders, in particular the reduction in large emission spikes can mitigate the risk to humans near fumigated areas.

Effect of Impermeable films on Fumigant Efficacy and Crop Yields

A reduced MBr:Pic (67:33 w/w) rate (196 kg/ha) applied under several VIF types and metalized mulch types controlled nutsedge (<21.5 plants/m²) better than a full rate (392 kg/ha) under HDPE (269 plants/m²) (Santos et al., 2007b). Reducing MBr:Pic rates by half (196 kg/ha)

under VIF provided similar nutsedge control as a full rate (392 kg/ha) under LDPE (Santos et al., 2005). In the same study, it was found that application rates could be reduced to 25% (99 kg/ha) of the standard application rate (392 kg/ha) under VIF without causing bell pepper yield losses. Under VIF, MBr:Pic (67:33 w/w) could be reduced by 25% while providing equal nutsedge control and by 50% to produce equal tomato yields as a full rate (393 kg/ha) (Hamill et al., 2008). A lower 1,3-D plus Pic application concentration (600 ppm) under VIF and metalized mulch resulted in good purple nutsedge control (<54 plants/m²), whereas a higher fumigant application concentration (1400 ppm) was needed for the same level of nutsedge control under HDPE (Santos et al., 2006a). Reduced rates of MBr:Pic 67:33 w/w (98 and 196 kg/ha) under two types of VIF resulted in similar nutsedge control and pepper yield as a commercially applied dose (392 kg/ha) under LDPE (Gilreath et al., 2005b). Rates of 1,3-D:Pic (61:35 w/w) could be reduced by 33% under TIF compared to HDPE, while maintaining similar weed control (yellow nutsedge, common purslane, and common chickweed) and strawberry fruit yield as a standard rate (392 kg/ha) of MBr under HDPE (Fennimore and Ajwa, 2011). These studies on MBr:Pic and 1,3-D:Pic have shown that application rates under VIF and TIF can be reduced to 25 - 57% of the application rate under HDPE and LDPE while maintaining fumigant efficacy against weeds and maintaining crop yields. Similar reductions may be possible with other fumigants if highly impermeable films are utilized.

Temperature Effect on Film Permeability

Film permeability is strongly affected by temperature. The mass transfer coefficient (cm/h) of fumigants across a 0.025 mm HDPE for various fumigants increased by a factor of 1.5 to 2.5 per 10°C increase in temperature from 20 to 40°C (Papiernik and Yates, 2002). In a calibration experiment under controlled conditions MBr emissions were measured to be 1/35 under an EVOH film (considered VIF at the time) compared to a conventional PE film (Wang et al., 1998a). MBr emission patterns were affected directly by temperature changes (increased emissions with increased temperatures and decreased emissions with decreased temperatures) in the calibration experiment for both films. However, under field conditions emissions did not seem to correlate with diurnal temperature changes, especially for the high barrier film. It was suspected that diffusion and degradation in the soil may play an equal or greater role in controlling MBr volatilization in the soil.

Gan et al. (1999) found that fumigant degradation for 1,3-D and MITC increased 5 to 12 times when temperature increased from 20 to 50°C. Chemical transformation of fumigants increased with increasing temperature, but the effect of temperature on microbial degradation was fumigant dependent. Temperatures $>30^{\circ}\text{C}$ suppressed microbial degradation of 1,3-D and stimulated degradation of MITC. Microbial degradation of fumigant was greatest in high organic matter soils and was higher for MITC than for 1,3-D.

Ma et al. (2001) found that, in addition to a temperature and microbial effect on fumigant degradation, initial fumigant concentration had an effect on the degradation rate constant. The MITC degradation rate constant was lower at high initial concentrations compared to low initial fumigant concentrations. This could have been caused by inhibitory effects of the fumigant on degrading microbial populations.

Guo and Gao (2009) found that the degradation rate of MeI was mainly due to soil organic matter and temperature. In a sandy loam soil with 4.3 g/kg of organic carbon, the calculated half-life of MeI applied at a rate of 49 mg/kg was 68 days at 10°C, 32 days at 20°C and 23 days at 30°C. They also found that MeI degradation in soil was rate dependent.

Degradation increased (41.1 to 7.4 day half life) as the application rate decreased (74 to 7 mg/kg). Optimal soil moisture for MeI to control weeds was 14% in sandy soils and greater efficacy was realized when soil temperatures were above 20°C (Zhang et al., 1998). In addition, MeI was much more effective than MBr under a range of soil moistures, temperatures, and soil textures.

Grafting

Merriam-Webster dictionary (2012) defines graft (verb) as: “to cause (a scion) to unite with a stock; *also*: to unite (plants or scion and stock) to form a graft”. Savvas et al. (2010) described grafting as “the union of two or more pieces of living plant tissue, which are forced to develop vascular connection and grow as a single plant”. According to Lee and Oda (2003) ancient books written in China in the 5th century and in Korea in the 17th century describe a technique of using multiple graftings to produce large gourd fruit. Grafting interspecific herbaceous plants to increase yield and manage disease was first reported in Japan during the late 1920s using a squash rootstock (*Curbita moschata* Duch.) to confer fusarium wilt resistance in watermelon production (Kubota et al., 2008 and Louws et al., 2010; King et al., 2010).

In North America, tube grafting is most commonly utilized for grafted tomato transplant production (Kubota et al., 2008). Tube grafting is accomplished by using an elastic plastic tube to hold together the scion and rootstock, which are cut at complementary 45° angles. Following grafting, transplants are placed under high humidity and low light intensity for 4 to 7 days to heal (Kubota et al., 2008). After healing, grafted transplants are placed in a greenhouse to finish healing and acclimate. Grafting should be performed below the cotyledons to prevent rootstock suckering. When grafting was performed below the cotyledons rootstock regrowth was 0%, however when grafting was done above the cotyledon rootstock regrowth was as high as 85% for some rootstocks (Bausher 2011). Rootstock regrowth of tomato can compete with the scions to lower desirable growth and yield.

The primary purpose for grafting tomatoes in Europe and the United States is to extend the harvest season in greenhouse production (King et al., 2010). When plants are grown in the soil in non-heated greenhouses disease resistance is the main reason for grafting, and in open field production grafting is done primarily to manage bacterial wilt (*Ralstonia solanacearum* (Smith) Yabuuchi et al.) (few resistant cultivars) or for brown root rot (*Colletotrichum coccodes* (Wallr.) Hughes) (no resistant cultivars) (King et al., 2010). The rationale behind grafting is to: 1) achieve resistances to soilborne diseases and nematodes, 2) increase yields and quality, and 3) improve the physiology of plants to make them suitable for harsh environments (Kubota et al., 2008; King et al., 2010).

Grafting has been shown to manage fungi (*Verticillium dahlia* race 1, *Verticillium albo-atrum*, *Fusarium oxysporum* f. sp. *lycopersici* race 0, 1 & 2, *Fusarium oxysporum* f. sp. *radicis-lycopersici*, *Sclerotium rolfsii*, *Pyrenochaeta lycopersici*, *Colletotrichum coccodes*, and *Rhizoctonia solani*), oomycetes (*Phytophthora* spp.), bacteria (*Ralstonia solanacearum*) and rootknot nematodes (*Meloidogyne* spp.) in tomato (Louws et al. 2010; King et al., 2008; Lee 1994).

Grafting on tobacco rootstock resulted in a 5% and 30% increase in total fruit weight of tomato cultivars Sweet and Elazig, respectively (Yasinok et al., 2009). Plant height of cultivar Big Red grafted on hybrid tomato He-Man was increased compared to the non-grafted control in an open field experiments (Khah et al., 2006). Furthermore, plant height was increased in a greenhouse experiment when grafted onto hybrid rootstock ‘Primavera’ compared to non-grafted

'Big Red'. Total fruit weight increased in the greenhouse experiment when plants were grafted on 'He-Man' compared to self grafted 'Big Red'. Physical properties (visual appearance and texture), flavor compounds, and health related compounds (desirable and undesirable) of fruiting vegetables can be impacted by grafting (Rouphael et al., 2010). Increased fruit yield, soluble solids content (SSC), and titratable acidity (TA) was reported when tomato cultivar Moneymaker was grafted onto 'Radja' rootstock under saline soil conditions (50 mM NaCl) compared to self grafted 'Moneymaker' (Flores et al., 2010). Higher tomato fruit quality (increased SSC and TA) was obtained using cultivar Kyndia grafted on processing cultivar UC82B under saline conditions (50 mM NaCl) compared to self grafted 'Kyndia' plants (Flores et al., 2010). In addition to increasing yields and fruit quality under saline conditions, recombinant inbred lines from *Solanum lycopersicum* × *Solanum cheesmaniae* used as rootstocks increased SSC and TA compared to self grafted commercial tomato hybrid 'Boludo' under non-saline conditions (Flores et al., 2010). Marketable tomato fruit increased by 30% per plant by grafting, however total vitamin C and phenolics decreased after grafting (Vrcek et al., 2011). Grafting has resulted in increased carotenoids in tomato (Davis et al., 2008). In addition, grafting can affect the period of flowering and harvest. Physiological interactions between rootstock and scion can affect water flow, nutrient uptake and translocation, plant growth and biomass (Martinez-Ballesta et al., 2010). Grafting should achieve optimal bundle connection between rootstock and scion to prevent problems associated with graft incompatibility. Tomato vegetative growth and fruit yield was decreased, while blossom end rot incidence on fruits, leaf chlorophyll concentrations, soluble solids and sugar contents was increased in plants grafted onto scarlet eggplant (*Solanum integrifolium* Poir.) compared to plants grafted on tomato (Oda et al., 1996). These symptoms indicate that the plants grafted on scarlet eggplant rootstock may have been in a state of water deficiency.

Several studies have shown increased tomato yield under saline conditions when grafting is used. Mechanisms of salt tolerance in grafted plants include salt exclusion in the shoot and retention of salt ions in the root, greater maintenance of potassium homeostasis, accumulation of compatible solutes and osmolytes, antioxidant defense systems and hormone mediated plant growth changes (Colla et al., 2010). Fruit yield was higher in the tomato cultivars Fanny and Goldmar when grafted onto the tomato hybrid rootstock 'AR-9704' at various salinity levels (0, 30, and 60 mM NaCl) (Fernandez et al., 2004). Concentrations of β -carotene and lycopene were greater due to grafting, but not salinity. Tomato cultivar Moneymaker grafted on rootstocks 'Pera' or 'Radja' improved yield compared to self grafted 'Moneymaker' when plants were grown at 50 mM NaCl, although grafting had no yield effect at lower concentrations (0 and 25 mM NaCl) (Martinez-Rodriquez et al., 2008). At 50 and 75 mM NaCl, plants grafted onto 'Radja', 'Pera', and hybrid Volgogradskij×Pera had yield increases of approximately 80% compared to the non-grafted cultivar Jaguar (Estan et al., 2005). The yield differences were related to the ability of the rootstocks to reduce ionic stress, because there was a negative correlation between fruit yield and leaf Na^+ or Cl^- concentrations. In addition to possessing salt tolerance, tomato plants (cultivar Belladonna) grafted on 'Beaufort', 'He-Man', and 'Registar' rootstocks were deficient in leaf Mg compared a self-grafted control in a grafting salinity experiment (Savvas et al., 2011). Tomato cultivar Hezuo903 grafted on the salt tolerant rootstock 'Zhezhen No.1' improved photosynthesis by increasing stomatal conductance and water use efficiency under saline conditions, maintained higher photochemical activity of photosystem II, increased antioxidant enzyme activity of catalase and enzymes involved in ascorbate-glutathione cycle and decreased lipid peroxidation, which increased tomato growth

under saline conditions compared to non-grafted and self-grafted plants (Yong et al., 2009).

Grafting can be used as a tool to improve tolerance of water stress (drought and flooding), organic pollutants and thermal stress (suboptimal and supraoptimal) (Schwarz et al., 2010). The effect of grafting on temperature stress has been investigated in several experiments. The impact of high-temperature stress was decreased in grafted tomato plants compared to non-grafted plants (Rivero et al., 2003a, Rivero et al., 2003b). Therefore, grafted plants that utilize rootstocks that are more tolerant to thermal stress can become more resistant to thermal stress. Under low temperature conditions tomato rootstocks had significant effects on vegetative growth, earliness, and fruit production (Zijlstra and Den Nijs, 1987). A high altitude accession of *Solanum habrochaites* Humb & Bonpl. (LA 1777) used as a rootstock also improved suboptimal temperature tolerance of a tomato scion (cultivar Moneymaker) compared to a self-grafted tomato control (Venema et al., 2008).

In certain Asian countries, many grafted vegetables are grown in commercial fields and greenhouses to manage soilborne diseases. Grafted plants now account for 81 and 54% of the vegetable acreage in Korea and Japan, respectively (Lee, 2003). These vegetables include watermelon, cucumber, oriental melons, muskmelons, tomatoes and eggplant. In these areas, grafted plants are used to deal with the problems associated with intensive vegetable production and the lack of crop rotation due to limited arable land (Kubota et al., 2008; Lee 1994). According to data reported by Lee (1994), there were 300 hectares of grafted tomatoes in open field production and 800 hectares in greenhouse production in Japan during the early 90's. In addition there were 40 hectares of grafted tomatoes in greenhouse production in Korea. The species of rootstocks commonly used for grafting tomatoes are *Solanum pimpinellifolium* (L.) Mill., *Solanum habrochaites* S. Knapp & D.M.Spooner, and *Solanum lycopersicum* (Lee, 1994).

Growing grafted vegetables in the United States, where land use is not intensive, is seldom practiced, but has recently been gaining popularity, partly due to the loss of methyl bromide, loss of quality disease free farm land and the increased restrictions of using soil fumigants (Lee, 1994 and King et al., 2010). Grafting cost, limited available rootstock information, limited grafting facilities, exemptions for MBr use, transportation costs, and legal issues have led to slow acceptance of grafting in the United States (King et al., 2010; Kubota et al., 2008). An economic analysis reported that the cost of grafted and non-grafted transplants were \$0.59 and \$0.13 in North Carolina, and \$1.25 and \$0.51 in Pennsylvania (Rivard et al., 2010a).

Grafting for the Management of Bacterial Wilt

Several studies have demonstrated the effectiveness of grafting to manage bacterial wilt in tomato. Grafting tomato (ASVEG10) on bacterial resistant eggplant (EG203) resulted in 0 to 2.8% wilted plants, grafting on resistant tomato (Hawaii 7996) resulted in 7 to 47% wilted plants and non-grafted tomato (ASVEG 10) led to 24 to 93% wilted plants in field experiments conducted at four sites (Lin et al., 2008). These rootstocks also performed well against bacterial wilt strains in preliminary greenhouse studies. Tomato cultivar Momotaro grafted onto *Solanum toxicarium* Rich. was completely resistant to five strains of bacterial wilt (Matsuzoe et al., 1993). Bacterial wilt susceptible cultivar Pusa Ruby grafted onto resistant tomato cultivar (CRA-66-Sel-A) and resistant eggplant (Dingra's Multiple Purple) increased plant survival (100% survival) in bacterial wilt infested soils compared to the non-grafted susceptible cultivar (0% survival) (Tikoo et al., 1979). The non-grafted resistant tomato cultivar also resulted in 100% plant survival, but the fruit was small and had green shoulders. Non-grafted heirloom tomato cultivar

German Johnson exhibited 75 to 79% bacterial wilt incidence in naturally infested soils (Rivard and Louws, 2008). However, ‘German Johnson’ grafted onto genotypes CRA 66 and Hawaii 7996 showed no bacterial wilt symptoms. Calcium concentration may further increase bacterial wilt resistance in grafted plants. Bacterial wilt populations in the xylem exudates from the scion of inoculated grafted plants decreased with increasing calcium concentrations in the growing solution (Yamazaki et al., 2000a). In another study, calcium uptake increased in plants grafted on the resistant cultivar (Hawaii 7998), however resistance was still attributed to rootstock and not calcium uptake (Yamazaki et al., 2000b). The authors concluded that calcium uptake differences by grafted tomatoes might not be related to the expression of the resistance to bacterial wilt. Bacterial resistant variety (BHN 446) had lower incidence of disease compared to fumigation (MBr and 1,3-D) (Driver and Louws, 2002). Hybrid rootstocks may also aid in increasing tomato fruit yield. A bacterial wilt grafting experiment on the Eastern Shore of Virginia indicated that a bacterial wilt susceptible commercial cultivar BHN 602 grafted on a hybrid tomato bacterial wilt resistant rootstock ‘RST-04-106’ produced higher yields (75619 kg/ha) than the non-grafted commercial cultivar alone (62610 kg/ha) (Freeman et al., 2009a).

Previous research has indicated that resistance to bacterial wilt in tomato does not prevent the pathogen from entering the host root system, but reduces its spread into the stem vasculature (Grimault and Prior, 1993; Prior et al., 1996). Bacterial wilt moves from vessel to vessel through degenerated pit membranes (Nakaho et al., 2000). Resistant rootstocks may limit bacterial movement by thickening pit membranes and/or accumulating electron dense materials in vessels and parenchyma cells (Nakaho et al., 2000). In resistant tomato cultivars, occlusion of colonized vessels by tyloses limited the spread of bacterial wilt (Grimault et al., 1994). Gums, cell wall breakdown, and modifications of the primary cell wall also contributed to bacterial wilt resistance or susceptibility in tomato (Grimault et al., 1994). In case of ‘Hawaii 7996’, a restriction in the movement of the pathogen from the protoxylem to other xylem tissues has been noted. Due to this restriction, the incidence of bacterial wilt on a susceptible scion ‘Ponderosa’ grafted onto ‘Hawaii 7996’ rootstock was significantly less compared to non-grafted and ‘Ponderosa’ grafted to a susceptible scion (Nakaho et al., 2004). Another study suggested the bacterial wilt resistance mechanism in tomato is an upregulation of an apical membrane antigen through salicylic acid induced defense signaling (Afroz et al., 2009). Rootstocks resistant to bacterial wilt are not necessarily resistant to nematodes and other major tomato diseases (Lee, 1994).

Grafting for Nematode Management

Studies have documented the ability of grafting on resistant rootstocks to manage root-knot nematode. In a greenhouse study, seven hybrid tomato rootstocks (PG76, Gladiator, MKT-410, Brigeor, 42851, 43965 and Big Power) planted directly into soil with high population densities of *Meloidogyne javanica* (Treub) Chitwood (2050 ± 900 second stage juveniles per 250 cm³ soil) showed high levels of resistance, one showed intermediate resistance (He-Man) and two were susceptible (Beaufort and Maxifort) (Cortada et al., 2008). Rootstocks ‘PG76’, ‘Gladiator’ and ‘MKT-410’ were consistently highly resistant to a *Mi*-avirulent population of *M. javanica* in two tests conducted in pots. Presence of the *Mi* locus in the tested hybrid rootstocks were confirmed using PCR co-dominant markers REX-1 and Mi23. In another study, three interspecific (*S. lycopersicum* × *S. habrochaites*) rootstocks (Big Power, Beaufort and Maxifort) reduced root-knot nematode (*M. incognita*) area under the disease progress curve and soil populations at first harvest (Rivard et al., 2010b). ‘Big Power’ was effective at reducing root-

knot nematode galling and soil populations at the last harvest. Marketable fruit yields were higher for the nematode resistant rootstocks compared to non-grafted and self grafted susceptible heirloom cultivar German Johnson. *M. javanica* gall ratings and eggs/gram root were lowest in the non-grafted *Mi* resistant tomato cultivar Monika, intermediate in nematode susceptible tomato cultivar Durinta grafted onto resistant tomato rootstock ‘SC 6301’ and highest in the non-grafted susceptible cultivar Durinta (Verdejo-Lucas and Sorribas, 2008). Resistance to *M. incognita* was observed when highly susceptible tomato ‘Kyouryokubeiju’ was grafted on *S. toxicarium* and *S. torvum* (Matsuzoe et al., 1993). Tomato cultivar Blitz grafted on rootstock ‘Hypeel45’ had less root-knot nematode (*M. incognita*) populations than ‘Blitz’ grafted on ‘Beaufort’ and both rootstocks had less galling than non-grafted ‘Blitz’ (Lopez-Perez et al., 2006). Both rootstocks resulted in higher yields than non-grafted ‘Blitz’. The *Mi* nematode resistance gene is known to break down in high soil temperatures (above 28°C) (Louws et al., 2010). At 24°C, tomato cultivar Astona RN F1, rootstock cultivars Vigomax and Beaufort, and *S. peruvianum* accessions PI 126443 and PI 270435 were resistant to *M. incognita* race 2 compared to susceptible cultivar Simita F1 (Devran et al., 2010). At 32°C, ‘Simita F1’, ‘Astona RN F1’, ‘Vigomax’ and ‘Beaufort’ were susceptible to *M. incognita* race 2, but PI 126443 and PI 270435 were resistant.

Effect of Grafting on Tomatine?

Grafting tomato onto jimson weed (*Datura stramonium* L.) was practiced on a limited scale in the United States to manage root-knot nematodes and was recommended to home gardeners (Kubota et al., 2008). However, grafting tomato to jimson weed was not done commercially possibly because of the potential to transport small amounts of tropane alkaloids to the fruits. In grafted plants of belladonna (*Atropa belladonna*) and angel’s trumpet (*D. innoxia*) alkaloid ratios (hyoscyne/hyoscyamine) were shown to be indicative of the rootstock used not the scion (James and Thewlis, 1952). Another study investigated alkaloid distribution (hyoscyamine) from grafts of black henbane (*Hyoscyamus niger* L), Egyptian henbane (*H. muticus* L.), *Scopolia anomala* (Lk.et Otto) Airy Shaw with strawberry ground cherry (*Physalis alkekengi*), tomato and potato (Warren Wilson, 1952a). Grafting tomato scions on *H. niger* rootstock resulted in 0.02% hyoscyamine in the leaves and 0.1% in the stems. Leaves, stems, and green fruit from *H. niger* scions were alkaloid free when grafted on tomato. *P. alkekengi* grafted on *H. muticus* gave assays of 1.01% hyoscyamine for the leaves and 3.52% for the stems. *H. muticus* grafted on tomato were alkaloid free. *P. alkekengi* grafted on *S. anomala* rootstock produced 0.39% hyoscyamine in the leaves and 1.07% in the stems. Potato (Ballydoon) grafted on *S. anomala* rootstock contained 0.25% hyoscyamine in the leaves and 0.24% in the stems. *S. anomala* grafted on *P. alkekengi*, potato, or tomato rootstocks were alkaloid free. Therefore, it appears the hyoscyamine is transported from the rootstock to the scion in grafted plants. There is also evidence that solanaceous alkaloids from jimson weed and belladonna were transported in a downward direction to tomato rootstock (Warren Wilson, 1952b; Warren Wilson, 1959).

No appreciable amounts of nicotine were detected in leaves or stems of tobacco scions when grown on tomato rootstock (Dawson, 1942). However, when tomato scions were grafted on tobacco rootstock small quantities of nicotine were found in the stems and fruit, while large quantities accumulated in the leaves. Nicotiana plants (*N. tabacum* L. cv. Maryland Robinson Medium Broadleaf tobacco, *N. tabacum* cv. Swarr-Hibshman Pennsylvania cigar tobacco, *N. tabacum* L. cv. Baur, *N. glauca* Grah. and *N. glutinosa* L.) grafted onto tomato rootstock produced appreciable amounts of nicotine and nornicotine (Jeffery and Tso, 1964). These results

indicate alkaloid synthesis is possible with the absence of roots from some alkaloid producing plants. The total amount of alkaloids found in the scion and rootstock were always lower than non-grafted tobacco plants. Tomato cultivars Elazig and Sweet grafted onto a low nicotine containing tobacco cultivar Samsun produced tomato fruits containing nicotine levels within acceptable ranges that were considered safe for consumption (Yasinok et al., 2009). It appears steroidal glycoalkaloids from grafted potato (α -solanine and α -chaconine) and tomato (α -tomatine) do not transport between rootstock and scion in these species (Roddick, 1982).

Glycoalkaloids are N-containing plant secondary metabolites (Friedman 2002). They are found in many solanaceous plants including eggplant, potato, and tomato. Glycoalkaloid-containing plants may use these compounds as chemical defenses against fungi, insects and animals. Glycoalkaloids resist pathogens by binding to cholesterol, disrupting cell membranes, and inhibiting cholinesterases (Friedman, 2002). Tomatine is a steroidal glycoalkaloid that naturally occurs in tomato. Tomatine which was discovered by Fontaine et al. (1948) consists of α -tomatine and dehydrotomatine. Tomatine consists of a hydrophilic part (a tetrasaccharide side chain), a hydrophobic part (a steroidal moiety) and a polar secondary amine group. α -Tomatine is a glycoside that has four carbohydrate residues (lycotetraose) attached to the 3-OH group of the aglycon tomatidine. Dehydrotomatine has the same carbohydrate side chain, only differing from α -tomatine by the presence of a double bond due to the removal of 2 H's at C-atoms 5 and 6 of ring B (aglycon tomatidenol). Immature green tomato fruit contain up to 500 mg α -tomatine/kg of fresh fruit (Friedman, 2002). α -Tomatine degrades as the fruit ripens. In mature red tomatoes, α -tomatine levels are ~5 mg/kg of fresh fruit weight (Friedman, 2002). Methods for detecting and quantifying tomatine include high-performance liquid chromatography (HPLC) with ultra violet (UV) detection and liquid chromatography with mass spectrometry (LCMS).

Tomatine appears to be much safer for human consumption than potato glycoalkaloids (solanine and chaconine) and other solanaceous alkaloids (Friedman 2002, Rick et al., 1994). There is widespread consumption of fried green tomatoes, pickled green tomatoes, and tomatillos without apparent side effects. The LD50 values (in mg/kg body weight) for α -tomatine in mice were 25-33.5 for intraperitoneal, 500 for oral, and >1000 for subcutaneous administration (Sackmann et al., 1959; Nishie et al., 1975). High tomatine contents are found in ripe cherry tomatoes *Solanum lycopersicum* var. *cerasiforme* indigenous to the northwest Andes in Peru (Rick et al., 1994). These fruit contain 500-5000 μ g α -tomatine/g dry weight of ripe fruit. Ripe fruit from these accessions exhibit bitter flavor. It appears locals consume this high tomatine tomato without indigestion, discomfort, or other manifestations of toxicity. Tomatine is stable at high temperatures and is not modified by cooking (Rick et al., 1994).

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Chapter 2 Grafting using hybrid rootstocks for management of bacterial wilt in field tomato production

Introduction

Fresh market tomato (*Solanum lycopersicum* L.) production is a vital part of the agricultural economy in Florida and Virginia. The fresh market tomato industries in Florida and Virginia rank 1st and 4th nationally, respectively, and have a combined farm gate value of over 583 million dollars (USDA-NASS, 2011). Bacterial wilt of tomato caused by the soilborne bacterium *Ralstonia solanacearum* Smith race 1 (biovar 1, phylotype II) is widely distributed in the southeastern United States and causes considerable economical losses under ideal conditions for disease incidence (Ji et al., 2007).

Several studies have demonstrated the effectiveness of grafting to manage bacterial wilt in tomato but all have used open pollinated breeding lines that are not widely available (Lin et al., 2008; Rivard and Louws, 2008). The level of bacterial wilt resistance in available hybrid lines is unknown. It is also unclear as to whether hybrid rootstocks would increase tomato fruit yield over open pollinated rootstocks. The objectives of this study were to 1) determine the level of bacterial wilt resistance present in newly developed hybrid rootstocks, 2) assess the ability of grafted plants to reduce bacterial wilt in tomato field production, and to 3) determine the effect of the grafted plants on tomato fruit yield.

Materials and Methods

Greenhouse experiments

A well-characterized *R. solanacearum* race 1 strain Rs 5 (race 1 biovar 1, phylotype II) (Ji et al, 2005, Ji et al., 2007) isolated from tomato in Quincy, FL was streaked on CPG agar plate and incubated at 28°C for 48 h. Pure mucoid colonies of the strains were suspended in sterile deionized water (dH₂O) and adjusted spectrophotometrically to OD_{600nm}=0.1, which corresponds to ~10⁸ CFU/ml. The aqueous *R. solanacearum* was further diluted 10-fold to adjust the bacterial population to 10⁷ CFU/ml. Seventy-five ml of a bacterial suspension containing 10⁷ CFU/ml of *R. solanacearum* was poured into plastic pots filled with 750 ml of soil-less potting medium (Sungro Metro-Mix 200 series; Sun Gro Horticulture Canada Ltd., BC, Canada). The potting medium was evenly mixed with a sterile glass rod after inoculation. The rootstocks 'Jjak Kkung' (Seminis Vegetable Seeds, St. Louis, MO), 'Cheong Gang' (Seminis Vegetable Seeds), 'RST-04-105-T' (DP seeds, Yuma, AZ), 'RST-04-106-T' (DP Seeds), 'Hawaii 7998' (Public breeding material, University of Florida), 'BHN 998' (BHN Seed, Immokalee, FL), 'BHN 1053' (BHN Seed), 'BHN 1054' (BHN Seed), and the scion 'BHN 602' (BHN Seed), were transplanted into pots inoculated with *R. solanacearum* at the 3- to 4-leaf stage. The initial population of the bacterium in the potting medium was in the range of 10⁵-10⁶ CFU/g. The pots were arranged in a randomized complete block design. The temperature inside the greenhouse ranged between a minimum of 18-20°C and maximum of 34-38°C during the duration of the experiments. Plants were irrigated daily with 100-150 ml of tap water to maintain a soil water capacity of 80-90%. Plants were monitored for 28 days for bacterial wilt symptoms. Disease severity was scored on a scale of 0-4; 0= no symptoms, 1= one leaf with symptoms, 2=two or three leaves wilted, 3= all except the top two or three leaves wilted, and 4=all leaves wilted or dead. Means were calculated by the formula $[\sum (\text{score} \times \text{number of plants scored}) / (\text{total number of plants} \times \text{highest score})] \times 100$ (Nakaho, 2004). Bacterial wilt was confirmed by plating of the extract from infected plant samples on semi-selective modified SMSA medium

(Engelbrecht, 1994) and also testing with Immunostrips (Agdia Inc., Elkhart, IN). Plants with no signs of wilting were also tested for the presence of latent infection by the above method. The greenhouse experiments were conducted three times with 7 plants per treatment in each trial.

Experimental treatments for field trials

In all trials the tomato variety ‘BHN 602’ was used as a scion for grafted treatments as well as the non-grafted and self-grafted control. All trials included non-grafted and self-grafted treatments. The 2009 trial in Virginia included two rootstocks; ‘RST-04-105-T’ and ‘RST-04-106-T’. Resistance levels exhibited by ‘RST-04-105-T’ in the 2009 trial as well as in un-replicated field trials (J.H. Freeman *unpublished data*) were poor and it was not included in subsequent field trials. The 2010 trial in Virginia included six rootstocks; ‘RST-04-106-T’, ‘Cheong Gang’, ‘Jjak Kkung’, ‘BHN 998’, ‘BHN 1053’, and ‘BHN 1054’. The spring 2010 trial in Florida included ‘RST-04-106-T’, ‘Cheong Gang’, ‘Jjak Kkung’, and ‘Hawaii 7998’. The fall 2010 Florida trial included ‘RST-04-106-T’, ‘Cheong Gang’, ‘Jjak Kkung’, ‘BHN 998’, ‘BHN 1053’, ‘BHN 1054’, and ‘Hawaii 7998’.

Transplant production and grafting for field trials

Seedlings were grafted utilizing a modified Japanese tube graft at the two-leaf stage (Rivard and Louws, 2008). Recent research has indicated that grafting in this manner resulted in rootstock re-growth (Bausher, 2011). Due to the vigor of the rootstocks used this type of growth would necessitate pruning on a bi-weekly basis, which would be unsuitable for commercial field production. Thus, all grafted treatments were grafted below the rootstock cotyledons to prevent rootstock re-growth. Seedlings were grown pre and post grafting in expanded polystyrene trays of the inverted pyramid design with cell size 4.4 x 4.4 x 6.3 cm. Soil-less media was used for the production of all transplants. After grafting was performed, seedlings were placed in a high humidity chamber with controlled temperature to heal the graft union (Rivard and Louws, 2008). After one week, seedlings were removed from the chamber and placed in a greenhouse for 10-14 days until transplanting. Due to grafting below the rootstock cotyledon, care was taken at planting to maintain the graft union above the soil line.

Field experiments

Four field trials were conducted in Florida and Virginia during 2009 and 2010. Two of these trials were conducted on a commercial tomato farm in Painter, VA during the spring of 2009 and 2010. Soil type was Bojac sandy loam with pH 6.2. Two trials were conducted at the University of Florida, North Florida Research and Education Center in Quincy, FL during the spring and fall of 2010. Soil type was Norfolk sandy loam with pH 6.3. Experimental plots at both locations consisted of non-fumigated raised beds covered with black polyethylene mulch for spring plantings and white on black polyethylene mulch for fall plantings. Bed dimensions in both studies were 20.32 cm tall by 76.2 cm wide. In Virginia, beds were spaced 1.83 m apart and plants were spaced 45.72 cm within the row. In Florida, beds were spaced 1.83 m apart and plants were spaced 50.8 cm within the row. Inorganic fertilizers were applied to experimental plots based on soil test results and cooperative extension recommendations for respective states (Kuhar et al., 2009; Olson et al., 2011). The Virginia field plots had a history of bacterial wilt and were not inoculated. The Florida field plots didn’t have a history of bacterial wilt. To ensure disease development in the Florida field trial, experimental plots were inoculated with *R. solanacearum* Rs 5 strain. The strain was cultured by the methods described above. Seventy-five ml of an aqueous solution containing 10^7 CFU/ml of *R. solanacearum* was poured in each plant hole one day prior to transplanting. This created an initial bacterial population in the range of 10^5 - 10^6 CFU/g of soil. Grafted seedlings were transplanted on 29 May 2009 and 30 April 2010 in

Virginia and 20 April 2010 and 11 August 2010 in Florida. Each entry in the Virginia and Florida trials consisted of 4 replications with 30 and 18 plants, respectively, in each replication. All experiments were arranged as randomized complete block design. Experimental plots were assessed weekly for presence of symptoms typical for bacterial wilt infection. Disease incidence was calculated as the percentage of plants that had completely wilted. The cause of the wilting was confirmed using *R. solanacearum* specific Immunostrips. Disease incidence data is presented as percent incidence as recorded just after the final harvest. Experimental plots were maintained throughout the season with standard crop protection practices for commercial tomato production (Kuhar et al., 2009, Olson et al., 2011). Twelve plants from the center of each plot were marked, and fruit was harvested from these plants at a mature green/early breaker stage and graded by USDA Grades (USDA, 1991). Two to three harvests were made for each trial, which is typical of commercial tomato production in both states.

Statistical analysis

The greenhouse studies and field studies were set-up in a randomized complete block design. The data was analyzed using ANOVA, and the means were compared using least significant difference. The analysis was performed with SAS (SAS version 9.1, SAS Institute Inc., Cary, NC).

Results

Greenhouse experiments

All the rootstocks showed significant reduction in bacterial wilt incidence as indicated by the percentage of plants wilted, and the disease severity compared to the susceptible scion 'BHN 602' (Table 2.1). 'RST-04-105-T' exhibited a higher disease severity than other rootstocks. 'Hawaii 7998' and 'Cheong Gang' were the most resistant rootstocks in the greenhouse studies. All rootstocks except 'BHN 998' had significantly higher latently infected plants than 'BHN 602'. The percentages of healthy plants (no symptoms, and no latent infection) were significantly lower in 'BHN 602' compared to all the rootstocks except 'RST-04-105-T'. Among the rootstocks, 'BHN 998', 'Hawaii 7998', 'Cheong Gang', and 'RST-04-106-T' had the highest percentage of healthy plants.

Field experiments

Rootstock had a significant effect on bacterial wilt incidence in three experiments and tomato fruit yield in all experiments (Tables 2.2-2.5). The greatest yield and least disease incidence was always observed in one of the grafted treatments on a resistant rootstock.

Virginia 2009

Disease incidence was low to moderate in this trial. Rootstock treatment did not have a significant effect on disease incidence and no non-grafted plants exhibited bacterial wilt symptoms. The entry with the greatest numerical disease incidence was the self-grafted, followed by 'RST-04-105-T', and 'RST-04-106-T' (Table 2.2). Despite disease development in the non-grafted treatment, plants grafted onto 'RST-04-106-T' produced significantly greater total marketable yields than other treatments (Table 2.2). There were no differences in fruit yield between the remaining treatments.

Virginia 2010

Disease incidence was severe in this trial and rootstock treatment had a significant effect on percent incidence as well as fruit yield in all size categories and total marketable. All rootstock treatments yielded significantly greater than the non-grafted and self-grafted treatments (Table 2.3). Plants grafted onto 'Jjak Kkung' yielded 50,123 kg·ha⁻¹ of marketable fruit which

was significantly less than plants grafted onto ‘RST-4-106-T’, ‘Cheong Gang’, ‘BHN 1054’, and ‘BHN 998’. Plants grafted onto ‘BHN 1053’ yielded similarly to all other rootstock treatments with respect to marketable yield. No yield data were obtained from the self-grafted treatment due to near complete mortality at harvest. Least disease incidence was exhibited in rootstock treatments ‘BHN 1054’, ‘Cheong Gang’, ‘BHN 998’, and ‘RST -04-106-T’. These treatments all exhibited 13% or less disease incidence at harvest. Disease incidence in these treatments was significantly less than all other treatments. An intermediate level of disease incidence was observed in plants grafted onto ‘BHN 1053’ at 43.5%. Disease incidence exhibited by self-grafted plants was 97%, which was similar to non-grafted plants and plants grafted to ‘Jjak Kkung’. However ‘Jjak Kkung’ had significantly higher yield compared to non-grafted and self-grafted entries.

Florida spring 2010

Although bacterial wilt incidence was low overall ($\leq 7.5\%$ incidence) in this trial, rootstock treatment significantly affected disease incidence and tomato fruit yield. Numerically, greatest fruit yield was obtained from plants grafted onto ‘RST-04-106-T’ followed by ‘Cheong Gang’ and ‘Jjak Kkung’ (Table 2.4). All rootstock treatments resulted in significantly less bacterial wilt incidence compared to the non-grafted and self-grafted treatments. The self-grafted treatment exhibited significantly less bacterial wilt incidence than the non-grafted treatment.

Florida fall 2010

Bacterial wilt incidence was severe in this trial and rootstock treatment had a significant effect on disease incidence as well as tomato fruit yield. Non-grafted and self-grafted treatments exhibited the greatest amount of bacterial wilt incidence, 93.8 and 93.9%, respectively (Table 2.5). Plants grafted onto ‘BHN 1053’ exhibited similar amounts of disease incidence. Rootstock treatment ‘Cheong Gang’ resulted in the least amount of disease incidence (28.4%) but was statistically similar to ‘BHN 998’, and ‘BHN 1054’. ‘Hawaii 7998’, ‘RST04-106-T’ and ‘Jjak Kkung’ had significantly greater disease incidence than ‘Cheong Gang’ but significantly less than the non-grafted and self-grafted treatments. The non-grafted and self-grafted treatments produced less than $400 \text{ kg}\cdot\text{ha}^{-1}$ of tomato fruit but were statistically similar to all treatments but ‘BHN 1054’, ‘BHN 998’, and ‘Cheong Gang’ at $47,728 \text{ kg}\cdot\text{ha}^{-1}$, $30,194 \text{ kg}\cdot\text{ha}^{-1}$, and $28,733 \text{ kg}\cdot\text{ha}^{-1}$, respectively.

Discussion

Tomato producers throughout the United States face losses in productivity due to soilborne pests. For decades these pests have been managed with soil fumigants, primarily methyl bromide. The use of methyl bromide is nearly finished in the United States due to its phase out under the Montreal Protocol (UNEP 2011). Even with the use of soil fumigants, management of some pathogens such as bacterial wilt caused by *R. solanacearum* can be limited (Chellemi et al., 1997; Enfinger et al., 1979). Producers are currently seeking alternatives to soil fumigation to manage soilborne pests in tomato. Grafting with resistant rootstocks may provide growers with a sustainable and eco-friendly practice for bacterial wilt management (Rivard and Louws, 2008). The current study is unique as it is the first study in United States, which included numerous hybrid rootstocks that have been extensively evaluated in two geographic locations for resistance to bacterial wilt of tomato.

The use of rootstocks with resistance to bacterial wilt had a significant effect on tomato fruit yield and bacterial wilt incidence in nearly all experiments during 2009 and 2010. In two

instances, bacterial wilt disease pressure was low yet rootstock still had a significant effect on marketable tomato fruit yield. In the trials where disease pressure was severe, rootstock treatments significantly reduced disease symptom expression and maintained an acceptable fruit yield. There was disparity between the results obtained in Virginia in 2010 and Florida fall 2010. Disease was severe in both instances, non-grafted and self-grafted controls exhibiting greater than 85% disease incidence, but symptom expression varied by location. In Virginia, rootstocks exhibited less disease than in Florida. Representative *R. solanacearum* strains from both locations were typed and found to be biovar 1, phylotype II, sequevar 7 (Ji et al., 2007; Caitlyn Allen, *unpublished data*), and hence strain is not likely the cause of differences between locations. The difference in bacterial populations in natural infested soil in Virginia and artificially infested soil (10^5 - 10^6 CFU/g) in Florida is a likely reason with the disease pressure comparatively higher in the Florida trial. Resistance levels exhibited by grafted plants in Virginia may be a more realistic expectation of how these rootstock cultivars would perform in large-scale production in naturally infested fields. The resistance exhibited by some of the rootstocks to bacterial wilt disease even under the highest disease pressure, as in the case of inoculated fields in Florida, is further proof supporting the use of grafting as a management option. Grafting of susceptible cultivars to resistant rootstocks is an effective management option available for growers who are currently abandoning fields infested with *R. solanacearum* due to the major economic losses associated with the occurrence of bacterial wilt. This will also significantly reduce the cost of moving production from location to location.

A recent study utilized two open pollinated tomato-breeding lines, 'CRA 66' and 'Hawaii 7996', as rootstocks for field production of heirloom tomatoes and reported no disease incidence in plants grafted to either rootstock while non-grafted and self-grafted entries exhibited 50-80 % incidence (Rivard and Louws, 2008). A similar rootstock, 'Hawaii 7998', was used in both Florida trials and exhibited 53.6% disease incidence when disease pressure was high. In Virginia in 2010, disease incidence was high and the most resistant rootstock exhibited 5% incidence and was statistically similar to rootstocks that exhibited 13% incidence. Differential resistance of various bacterial wilt resistant breeding lines to *R. solanacearum* strains have been previously studied, which indicated that 'Hawaii 7996' and 'Hawaii 7998' were the two most stable resistant lines tested (Lin et al., 2008). However, these lines were not completely resistant to all strains they were challenged with. This may explain the disparity between the results presented by Rivard and Louws (2008) and the results presented here.

There was some indication from the Florida experiments that there may be a yield advantage by using hybrid rootstocks. In the spring when disease pressure was low, several entries grafted to hybrid rootstocks produced fruit yield that was numerically, but not significantly greater than plants grafted to 'Hawaii 7998'. This trend was more evident in the fall when plants grafted to 'Hawaii 7998' yielded significantly less than plants grafted to 'BHN 1054' while disease incidence was similar. Fruit yield from plants grafted to 'Hawaii 7998' was statistically similar to all other hybrid rootstock entries but produced much lower fruit yield than plants grafted to 'BHN 998' and 'Cheong Gang'. This may be due to complex factors such as bacterial populations within the stem or it may be due to rootstock vigor. In 2009, plants grafted to 'RST-04-106-T' yielded significantly greater than non-grafted 'BHN 602', which was exhibiting no disease symptoms. In similar experiments conducted in North Carolina marketable yields of 'German Johnson' grafted onto 'Hawaii 7996' and 'CRA 66' were less than 22,000 kg·ha⁻¹ (Rivard and Louws, 2008). Although a different scion was used in the current experiments, tomato fruit yield was much greater. In addition, experiments comparing non-

grafted and self-grafted 'German Johnson' to 'German Johnson' grafted onto hybrid rootstocks 'Maxifort' and 'Robusta' had greater marketable fruit yields of up to 65,000 kg·ha⁻¹ but no significant difference between the non-grafted, self-grafted, and hybrid rootstock entries (Rivard and Louws, 2008). A more recent study showed significantly greater yield with hybrid rootstocks over non-grafted and self-grafted entries but these trials were conducted in areas with significant root-knot nematode (*Meloidogyne* spp.) populations (Rivard et al., 2010a). The effect of hybrid versus open pollinated rootstocks on tomato fruit yield remains questionable, but is an area that needs further investigation. Another area with very little information is the effect of grafted plants with resistant rootstocks on the long-term populations of *R. solanacearum* in the soil. Our research group's preliminary greenhouse studies have indicated that there was no significant drop in the pathogen population in the potting medium of healthy and latently infected rootstocks from initial inoculum levels during the 28 day period of the experiment (Paret, M. L., *unpublished data*). Further field studies are in progress to examine long-term effects of grafting on *R. solanacearum* populations.

One of the restrictions in commercialization of grafting for open field tomato production right now is the lack of major suppliers of grafted tomatoes at affordable costs. A recent economic analysis (Rivard et al., 2010b) has determined that cost of a single grafted plant and non-grafted plant were at \$0.59 and \$0.13 in North Carolina, and \$1.29 and \$ 0.51 in Pennsylvania, respectively. In Florida, greenhouse tomato growers have been purchasing grafted plants from Canada costing approximately \$1.00-1.75 per grafted plant. Even though grafting requires higher initial investment for transplants, it significantly reduces disease incidence and provides significant increase in marketable yield as indicated in our current study. This could offset any additional expenses that growers may have to spend for purchasing/ producing grafted plants. Grafted plants also performed very well in non-fumigated soil. Elimination of the cost of soil fumigation could also make the use of grafted plants more economically feasible.

Conclusions

These studies illustrate the benefits of grafting susceptible tomato scions onto resistant hybrid rootstocks when planted into soils heavily infested with *R. solanacearum*. Disease incidence was greatly reduced and tomato fruit yield was maintained at levels acceptable to commercial producers. These data indicate that several commercially available hybrid rootstocks have high levels of bacterial wilt resistance. 'Cheong Gang', 'BHN 1054', and 'BHN 998' were the most adapted rootstocks with respect to bacterial wilt resistance and resulting tomato fruit yield.

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Table 2.1. Resistance of tomato rootstocks ('Jjak Kkung', 'Cheong Gang', 'RST-04-105-T', 'RST-04-106-T', 'Hawaii 7998', 'BHN 998', 'BHN 1053', 'BHN 1054') and scion ('BHN 602') to the bacterial wilt strain Rs 5 from tomato in Quincy, Florida. Experiments were performed at the University of Florida North Florida Research and Extension Center in Quincy, FL. Plants at the 3- to 4-leaf stage were transplanted into pots inoculated with the *R. solanacearum* strain. The initial population of the bacterial pathogen was in the range of 10^5 - 10^6 CFU/g of potting medium. The percentage of plants wilted, latently infected, and healthy, and the disease severity index are given.

Entry	Percentage plants ^z (%)			Diseases Severity Index ^x
	Wilted	Latently Infected	Healthy	
Jjak Kkung	19.05 c ^y	38.10 a	42.86 ab	14.88 cd
Cheong Gang	14.29 c	33.33 ab	52.38 a	10.27 cd
RST-04-105-T	47.62 b	28.57 ab	23.81 bc	45.24 b
RST-04-106-T	19.05 c	28.57 ab	52.38 a	17.86 cd
Hawaii 7998	9.52 c	28.57 ab	61.90 a	7.14 d
BHN 998	19.05 c	19.05 bc	61.90 a	16.67 cd
BHN 1053	28.57 bc	28.57 ab	42.86 ab	26.19 c
BHN 1054	23.81 c	33.33 ab	42.86 ab	20.24 cd
BHN 602	85.71 a	4.76 c	9.52 c	80.95 a
LSD (0.05)	20.00	17.65	21.61	17.24
<i>P</i> > <i>F</i>	<0.0001	0.0365	0.0012	<0.0001

^z A total of 21 plants were tested (7 plants per rootstock/scion in a experiment and the experiments were conducted 3 times).

^y Column means followed by the same letter are not significantly different at $P \leq 0.05$ based on Least Significant Difference (LSD)

^x Disease severity index was scored on a scale of 0-4; 0= no symptoms, 1= one leaf with symptoms, 2=two or three leaves wilted, 3= all except the top two or three leaves wilted, and 4= all leaves wilted or dead and calculated by the formula $[\sum (\text{score} \times \text{number of plants scored}) / (\text{total number of plants} \times \text{highest score})] \times 100$ (18).

Table 2.2. Bacterial wilt incidence and yield of ‘BHN 602’ non-grafted or grafted onto rootstocks for bacterial wilt resistance in a field naturally infested with *R. solanacearum* in Painter, VA. The experiment was conducted in spring 2009.

Entry ^y	Fruit yield (kg·ha ⁻¹)			Total marketable	Bacterial wilt incidence (%)
	Medium	Large	Extra large		
Non-grafted	4,370	18,004	40,236 ab ^z	62,610 b	0.0
RST-04-106-T	5,556	22,034	48,027 a	75,619 a	1.5
RST-04-105-T	7,783	20,930	26,508 b	55,312 b	11.2
Self-grafted	4,107	16,689	39,768 ab	60,564 b	18.2
LSD (0.05)	ns	ns	13,849	12,276	ns
<i>P</i> > <i>F</i>	0.2128	0.4465	0.0396	0.0253	0.1133

^z Column means followed by the same letter are not significantly different at $P \leq 0.05$ based on Least Significant Difference (LSD). ns = not significant

^y Each entry consisted of 4 replications with 30 plants in each replication, and the experiment was arranged as a randomized complete block design.

Table 2.3 Bacterial wilt incidence and yield of ‘BHN 602’ non-grafted or grafted onto rootstocks for bacterial wilt resistance in a field naturally infested with *R. solanacearum* in Painter, VA. The experiment was conducted in spring 2010.

Entry ^y	Fruit yield (kg·ha ⁻¹)			Total marketable	Bacterial wilt incidence (%)
	Medium	Large	Extra large		
BHN 1054	5,420 a ^z	16,371 ab	58,158 ab	79,950 a	5.0 c
Cheong Gang	4,113 ab	14,211 bc	60,605 a	78,928 a	6.5 c
BHN 998	4,648 a	14,013 bc	55,645 ab	74,306 a	10.5 c
RST-04-106-T	5,136 a	19,176 a	56,139 ab	80,451 a	13.0 c
BHN 1053	2,459 b	9,852 c	46,551 ab	58,863 ab	43.5 b
Jjak Kkung	4,357 a	9,954 c	35,813 b	50,123 b	56.0 ab
Non-grafted	474 c	1,938 d	13,959 c	16,371 c	85.5 ab
Self-grafted	0 c	0 d	0 c	0 c	97.0 a
LSD (0.05)	1,855	4,390	20,705	24,075	21.1
<i>P</i> > <i>F</i>	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

^z Column means followed by the same letter are not significantly different at $P \leq 0.05$ based on Least Significant Difference (LSD). ns = not significant

^y Each entry consisted of 4 replications with 30 plants in each replication, and the experiment was arranged as a randomized complete block design.

Table 2.4. Bacterial wilt incidence and yield of ‘BHN 602’ non-grafted or grafted onto rootstocks for bacterial wilt resistance in a field artificially inoculated with *R. solanacearum* . Experiments were performed during spring 2010 at the University of Florida North Florida Research and Extension Center in Quincy, FL.

Entry ^y	Fruit yield (kg·ha ⁻¹)			Total marketable	Bacterial wilt incidence (%)
	Medium	Large	Extra large		
RST-04-106-T	4,022	10,065 b ^z	40,840	54,927 a	0.0 c
Cheong Gang	4,171	11,895 a	36,129	52,195 a	0.0 c
Jjak Kkung	4,575	10,170 ab	36,049	50,794 a	0.0 c
Hawaii 7998	6,019	9,682 bc	30,164	45,865 ab	0.0 c
Self-grafted	5,162	8,179 b	33,532	46,874 ab	2.7 b
Non-grafted	3,589	8,567 bc	28,263	40,419 b	7.5 a
LSD (0.05)	ns	1728.4	ns	9058.6	2.4
<i>P</i> > <i>F</i>	0.1147	0.0041	0.1160	0.0428	<0.0001

^z Column means followed by the same letter are not significantly different at $P \leq 0.05$ based on Least Significant Difference (LSD). ns = not significant

^y Each entry consisted of 4 replications with 18 plants in each replication, and the experiment was arranged as a randomized complete block design.

Table 2.5. Bacterial wilt incidence and yield of ‘BHN 602’ non-grafted or grafted onto rootstocks for bacterial wilt resistance in a field artificially inoculated with *R. solanacearum* . Experiments were performed during fall 2010 at the University of Florida North Florida Research and Extension Center in Quincy, FL.

Entry ^y	Fruit yield (kg·ha ⁻¹)				Bacterial wilt incidence (%)
	Medium	Large	Extra large	Total marketable	
Cheong Gang	3,026 ab ^z	7,455 ab	18,293 abc	28,733 ab	28.4 d
BHN 998	2,663 ab	5,661 b	21,870 ab	30,194 ab	40.0 cd
BHN 1054	3,929 a	11,313 a	32,486 a	47,728 a	40.6 cd
Hawaii 7998	1,311 bc	2,877 bc	7,595 bc	11,784 bc	53.6 bc
RST-04-106-T	1,320 bc	2,916 bc	7,160 bc	11,395 bc	57.8 bc
Jjak Kkung	1,385 bc	2,264 bc	3,785 bc	7,434 bc	67.9 b
BHN 1053	1,505 bc	3,060 bc	10,494 b	15,059 bc	76.1 ab
Non-grafted	102 c	75 c	199 c	376 c	93.8 a
Self-grafted	43 c	73 c	117 c	232 c	93.9 a
LSD (0.05)	2142.4	5374.6	19096	25914	24.7
<i>P</i> > <i>F</i>	0.0136	0.0040	0.0241	0.0132	<0.0001

^z Column means followed by the same letter are not significantly different at $P \leq 0.05$ based on Least Significant Difference (LSD)

^y Each entry consisted of 4 replications with 18 plants in each replication, and the experiment was arranged as a randomized complete block design.

Chapter 3 Grafting to Manage Root-Knot Nematode in Tomato

Introduction

Root-knot nematodes (*Meloidogyne* spp.) can cause major yield losses in tomatoes (Lopez-Perez et al., 2006), specifically in fresh market tomato in the southeastern United States (Rivard et al. 2010). Grafting with interspecific rootstocks has proven to effectively reduce root-knot nematode galling and soil populations while maintaining tomato productivity (Rivard et al., 2010). Certain tomato and tomato interspecific hybrid rootstocks are known to have resistance or tolerance to root-knot nematode species (*Meloidogyne incognita*, *M. arenaria*, *M. javanica*, and *M. hapla*) (Louws et al., 2010). There are differences in nematode resistance in *Mi*-gene-resistant tomato cultivars (Lopez-Perez et al., 2006).

The root-knot nematode resistant gene *Mi* is highly effective in many conditions, however it does not confer resistance at high soil temperature and *Mi*-virulent nematode isolates have been identified (Williamson, 1998). New genes (*Mi*-1 to *Mi*-9) have been identified for resistance to virulent strains (Williamson et al., 2010) and heat stable resistance at higher temperatures (Williamson et al., 2010; Wu et al., 2009). Only *Mi*-1 has been successfully used in tomato breeding in the past, new elite breeding lines have been identified for heat-stable resistance to southern root-knot nematode and other sources are wild species (*S. peruvianum*) that do not normally cross with cultivated tomato (Williamson et al., 2010; Wu et al., 2009).

Nematicides have been used to successfully suppress nematodes, but nematode resistant hosts are preferable because of the expense and environmental toxicity of nematicides (Williamson and Kumar, 2006). Furthermore, current management measures in intensive agricultural systems are heavily reliant on nematicides (such as methyl bromide fumigation), but alternative strategies are needed as effective chemicals are withdrawn from use (Fuller et al., 2008). Grafting can be effectively used to manage root-knot nematode in organic tomato production (Kaskavalci et al., 2009).

Materials and Methods

An experiment was performed in the fall of 2011 to determine the resistance of hybrid tomato rootstocks to root-knot nematode. Treatments were non-grafted 'BHN 602' (BHN Seed, Immokalee, FL), 'BHN 602' (BHN Seed) grafted on 'RST 106' (DP seeds, Yuma, AZ), 'BHN 602' (BHN Seed) grafted on 'BHN 998' (BHN Seed) and 'BHN 602' (BHN Seed) grafted on 'BHN 1054' (BHN Seed). Plants were tube grafted (described by Kubota et al., 2008) below the rootstock cotyledon, which has been shown to prevent rootstock shoot regrowth (Bausher, 2011). Experimental plots were arranged as a randomized complete block design with four replications. Experiments were conducted at the Virginia Tech Eastern Shore Agricultural Research and Extension Center (ESAREC) in Painter, VA. Soil type at ESAREC is a Bojac sandy loam (Thermic Typic Hapludults) with 59% sand, 30% silt, and 11% clay with pH ranging from 6.2 to 6.5 and organic matter content of 0.50 to 0.75%.

Yellow summer squash seed was planted during the spring of 2010. The soil at the base of the squash plants were inoculated with approximately 5000 root-knot nematode eggs/plant one month after emergence. The squash crop was maintained as a host crop for the nematodes following current production guidelines (Wilson et al., 2012). After the growing season the squash plants were mowed to the ground with a tractor-mounted rotary mower. Grafted and non-grafted tomato seedlings were then transplanted into the same beds to evaluate root-knot nematode resistance. The tomato crop received 224 kg/ha N and was grown following the

current production guidelines for staked, polyethylene mulched fresh market tomato in Virginia (Wilson et al., 2012).

Data Collection

Root-knot galling ratings and tomato yields were recorded. Tomatoes were harvested once and graded into USDA size categories (USDA, 1991). The plastic mulch was removed prior to assessing nematode galling. Ten plants from each plot were then carefully dug using shovels. Root systems from excavated plants were then carefully rinsed to remove soil. Washed root systems were rated for root-knot galling using a root gall index (RGI), which is a rating from 0 - 10 based upon galling severity (0 = complete and healthy root system with no infestation, 10 = plant and roots are dead) (Zeck, 1971).

Statistical Analysis

Analysis of variance (ANOVA) was performed on root-knot gall ratings and tomato yields. Significant differences between treatment means were determined using Duncan's multiple range test at $P \leq 0.05$.

Results

All the nematode resistant hybrid rootstocks had lower RGI than the susceptible non-grafted 'BHN 602' (Table 3.1). There were varying levels of root-knot nematode resistance between rootstocks. Plants grafted on 'RST 106' had the lowest RGI, followed by 'BHN 998', and 'BHN 1054'. 'BHN 1054' had the highest RGI among rootstocks claiming root-knot nematode resistance. There were significant differences in tomato yields between treatments for all yield categories, although yields were very low (Table 3.1). All treatments grafted on resistant rootstocks had similar marketable yields. In addition, plants grafted on 'RST 106' and 'BHN 998' had higher marketable tomato yields than the non-grafted 'BHN 602'.

Discussion

Yields for the nematode grafting experiment were very low due to a variety of factors. Seedlings were planted late during the late summer, which led to sub optimal growing temperatures late in the season. In addition, a hurricane passed through the area soon after the transplanting which severely damaged many plants. Lastly, plants were only harvested once since a freeze event prevented a second harvest.

Results indicate different levels of nematode resistance between tested rootstocks. Similar results have been found in other studies where varying levels of nematode resistance in grafted tomato rootstocks and *Mi* resistant tomato cultivars have been documented (Cortada et al., 2008; Rivard et al., 2010; Verdejo-Lucas and Sorribas, 2008; Lopez-Perez et al., 2006). In addition, soil temperatures may have been higher than optimum under the plastic mulch when the trial was established in late summer. The tomato plants were double cropped into black mulch that was used during a spring squash crop. Although the plastic was coated with a white calcium spray, it may not have lowered temperatures to optimal levels. Activity of the *Mi* gene for nematode resistance in tomato is known to break down at high soil temperatures (above 28 °C) (Louws et al., 2010; Devran et al., 2010).

Conclusions

Despite low yields there was a marked reduction in galling caused by root-knot nematodes and an increase in yield due to resistant rootstocks. The two rootstocks that had the lowest gall rating ('RST 106' and 'BHN 998') had the highest marketable yields. These

rootstocks also performed well in the bacterial wilt trial. Identifying rootstocks that have resistance to multiple soilborne pests will benefit open field tomato producers. Future studies should be conducted to confirm these results and ascertain heat stable nematode resistant rootstocks for grafted tomato.

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Table 3.1. Root Gall Index and tomato yield for non-grafted or grafted tomato plants in a root-knot nematode infested field. Experiments were performed during fall 2011 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA.

Treatment ^y	Gall Rating	Yield (kg/ha)			
		Medium	Large	Extra-Large	Marketable
Non-grafted 602	6.9 a ^z	474 b	996 b	725 b	2195 b
602/BHN 1054	4.5 b	1382 a	1782 b	1687 ab	4852 ab
602/BHN 998	1.5 c	1525 a	3009 a	1389 b	5922 a
602/RST 106	0.5 d	1443 a	2040 ab	3544 a	7027 a

^z Column means followed by the same letter are not significantly different at $P \leq 0.05$ based on Duncan's multiple range test.

^y Each treatment consisted of 4 replications with 20 plants in each replication, and the experiment was arranged as a randomized complete block design.

Chapter 4 Retention of Shank Applied Dimethyl Disulfide by Virtually and Totally Impermeable Film

Introduction

Virginia ranked 3rd or 4th in the United States for harvested fresh tomato (*Solanum lycopersicum* L.) acreage (1821 to 1942 ha) and generated approximately 48 to 63 million dollars in production value per year from 2009 to 2011 (USDA-NASS, 2012). Fresh market tomato is the number one vegetable commodity in Virginia. Approximately 90% of the tomato area in the state is on the Eastern Shore (Wimer et al., 2009). The use of methyl bromide (MBr) has been instrumental for the management of soil borne pests in tomato production. However, MBr was found to deplete stratospheric ozone and has been incrementally phased out under the Montreal Protocol on Substances that Deplete the Ozone Layer (UNEP, 2006).

Dimethyl disulfide (DMDS) is a chemical that has been registered as a pre-plant soil fumigant in tomato, pepper, eggplant, cucurbit crops, and strawberries grown in plasticulture for the management of nematodes, weeds, and soilborne plant pathogens. It has been shown to be efficacious for the management of several pest species including yellow nutsedge, purple nutsedge, large crabgrass, *Amaranthus* spp., *Phythium* spp. in several vegetable crops such as tomato, cantaloupe, and strawberry (Olson and Rich, 2007; Culpepper et al., 2008; Welker et al., 2006; Othman et al., 2010; Welker et al., 2007; Lopez-Aranda et al., 2009; Garcia-Mendez et al., 2008; De Cal et al., 2004). DMDS is currently only labeled for use with VIF and metalized mulch. TIF has the potential to increase DMDS retention and pest control efficacy, which may allow for lower fumigant application rates and may reduce emissions. A potential drawback to TIF may be an increase in plant back period necessary to prevent phytotoxicity. The plant back period for Paladin under VIF and metalized mulches is determined by the mean daily minimum soil temperature at 20 cm depth. Currently the plant back period is 21 to 42 days after fumigation (DAF) for VIF film depending on soil temperature. The plant back period is 28 DAF for soil temperatures between 16 and 21.1°C and 21 DAF for soil temperatures 21.7 °C and higher. It is important to understand the effects of TIF mulch on DMDS use at various rates during different production seasons. The objective of this study was to determine DMDS retention by VIF and TIF for open field spring and fall seasons in Virginia.

Materials and Methods

DMDS fumigant retention experiments were conducted at the Virginia Tech Eastern Shore Agricultural Research and Extension Center (ESAREC) in Painter, VA during the fall of 2009, spring and fall of 2010, and spring and fall of 2011. Soil type at ESAREC is a Bojac sandy loam (Thermic Typic Hapludults) with 59% sand, 30% silt, and 11% clay with pH ranging from 6.2 to 6.5 and organic matter content of 0.50 to 0.75%. Soil was cultivated to a depth of 30 cm prior to fumigation. If necessary, overhead sprinkler irrigation was used to bring soil moisture to between 50 and 75% field capacity. A 79:21 w/w formulation of DMDS:chloropicrin (Pic) (United Phosphorus Inc., King of Prussia, PA, USA) fumigant was shank applied using a single row combination bed press with bed dimensions 76 cm wide and 20 cm high with three back swept shanks. Shanks were 20 cm long and fumigant was released at the bottom of the shank. Experimental plots were 24 m long with a between row spacing of 1.8 m. Fumigant was applied on 12 July, 2009, 8 April, 2010, 14 June, 2010, 2 May, 2011, and 14 June, 2011. Soil temperature at 20 cm prior to fumigant application was 24°C in the fall of 2009,

13°C in the spring of 2010, 23°C in the fall of 2010, 21°C in the spring of 2011, and 24°C in the fall of 2011.

Two mulches were used to compare DMDS retention. During all experiments, either a black (spring) or white on black (fall) formulation of Blockade® VIF (Berry Plastics Corp., Evansville, IN, USA) embossed polyethylene mulch with thickness 0.03 mm containing a nylon barrier was used. The TIF mulch used was a black (spring), or white on black (fall) Vaporsafe® TIF (Raven Industries Inc., Sioux Falls, SD, USA) polyethylene mulch with 0.05 mm thickness containing an EVOH barrier. A white on black formulation of TIF was not available during the fall of 2009, so the mulch was painted white the day after application with white exterior latex paint. Comparative temperature readings were taken between the painted mulch and factory white VIF mulch and found to be statistically similar (data not presented).

The labeled rate for DMDS:Pic application under VIF film in tomatoes to control nutsedge is 561 L/ha (642 kg/ha) (broadcast). Furthermore, Paladin is not yet labeled for use with TIF. The fall 2009 experiment included a standard rate of DMDS:Pic 79:21 w/w (468 L/ha (535 kg/ha broadcast)) under VIF and TIF, a high rate (561 L/ha (642 kg/ha broadcast)) under VIF, and two reduced rates (281 L/ha (321 kg/ha broadcast) and 374 L/ha (428 kg/ha broadcast)) under TIF. The experiment was repeated in the spring and fall of 2010 and 2011 with the addition of a 187 L/ha (214 kg/ha broadcast) rate under TIF. Fumigant rates were adjusted with a Siemens® (Siemens Corporation, New York, NY, USA) flow meter using a float (float code 708) rated for 3.5 L/min of DMDS:Pic 79:21 at 100% flow, inserting different sized TeeJet® (TeeJet Technologies, Wheaton, IL, USA) flow regulators (orifice plates), and by varying tractor speed, as described by Gilreath et al. (2005). Experimental plots were arranged as a randomized complete block design with four replications.

Data Collection

Soil temperature and fumigant persistence were collected for these experiments. Fumigant persistence was measured using a MiniRAE 3000 volatile organic compound (VOC) detector (RAE Systems, San Jose, CA, USA). The VOC detector uses a lamp and an ultraviolet (UV) light to ionize compounds that can be counted by a photo ionization detector (PID) (Noling et al., 2008). A MiniRAE VOC meter was used to measure 1,3-D plus Pic retention under TIF in a similar study (Fennimore and Ajwa, 2011). We used a 9.8 electron volt (eV) ionization energy lamp which does not have a response to Pic, therefore only DMDS was detected by the lamp. The maximum readable concentration for the MiniRAE 3000 is 3,000 ppm DMDS. A particle filter was used to prevent soil particles and moisture from entering the meter. VOC readings were taken in the soil under the plastic every other day after the fumigants began to dissipate until planting. Sampling earlier when fumigant concentrations are extremely high can damage internal sensors in the equipment and confound subsequent readings. Therefore, periodic samples were taken to determine when fumigant concentrations began decreasing and then sequential sampling was initiated. VOC sampling was performed during the afternoon on sampling dates. Samples were taken equidistant from the bed center and the bed shoulder. A 1.3 cm diameter round wooden dowel was inserted vertically to a depth of 10 cm. The dowel was removed and the probe was immediately inserted into the headspace. A seal was formed between the mulch and particle filter and readings continued until values stabilized or decreased and maximum values were recorded. Three sub-samples were taken from each plot and averaged. Sampling holes were covered with Poly Tarp Tape (BAC Industries Inc., Minneapolis, MN, USA) after measurement to prevent gas escape. Subsequent samples were taken from separate sampling holes.

Soil temperatures under the plastic were measured using two S-TMB-M002 12-bit Temperature/RH Smart Sensors (Onset Computer Corp., Bourne, MA, USA). These sensors were attached to a HOBO micro station data logger (Onset Computer Corp., Bourne, MA, USA), which recorded and stored the data. Temperatures were measured within the fumigated zone at depths of 5 cm and 20 cm. The data logger measured temperature every 30 seconds and averaged the readings every two minutes. Temperatures at both depths are reported for 8 am and 3 pm. These represent the daily maximum and minimum temperatures.

Statistical Analysis

The retention data was transformed using a log transformation. Analysis of variance (ANOVA) was performed on transformed data that was sorted by day after fumigation (DAF). When appropriate, significant differences between treatment means were separated using Duncan's multiple range test at $P \leq 0.05$.

Results

Fall 2009

Soil temperatures ranged between 21 and 40°C during the period from fumigation until planting (Fig 4.1). Average high soil temperatures at 3 pm were 35°C at 5 cm and 30°C at 20 cm. Low temperatures at 8 am averaged 25°C at 5 cm and 27°C at 20 cm. During this experiment the 468 L/ha rate under VIF was not sampled. In subsequent experiments it was sampled in addition to the 561 L/ha rate and DMDS concentration was found to be statistically similar (Fig 4.4, Fig 4.6, Fig 4.8, Fig 4.10). Initially, the TIF mulch retained DMDS at a greater concentration than the VIF mulch regardless of rate (Fig 4.2). Approximately three weeks after fumigation there were significant differences in fumigant retention between treatments. In general, the 468 L/ha rate under TIF was retained at a greater concentration than the 374 L/ha rate under TIF, followed by the 281 L/ha rate under TIF and the 561 L/ha rate under VIF. In this experiment, a reduced rate (281 L/ha) under TIF maintained greater fumigant concentrations than a high rate (561 L/ha) under VIF for 22 days. Four weeks after fumigant application there were differences in fumigant concentration between treatments, however these concentrations were very low and were likely biologically insignificant. The labeled plant back period for DMDS at the recorded average low soil temperatures at 20 cm under specified VIF films was 21 DAF (Table 4.1). At 21 DAF the typical DMDS concentrations under VIF for the standard rate was 25 ppm or lower. Therefore, we considered 25 ppm DMDS as a threshold value for safe plant back in tomato based on our observations. The VIF treatment (561 L/ha) contained very low concentrations of fumigant (≤ 5 ppm DMDS) at the labeled plant back period and could be safely planted. The treatments under TIF contained greater fumigant concentrations (39 - 3000 ppm DMDS) at 21 DAF. Fumigant concentrations under TIF did not drop below 25 ppm DMDS until 22 DAF for the 281 L/ha rate and 28 DAF for the 374 and 468 L/ha rates.

Spring 2010

Soil temperatures ranged between 9 and 39°C during the period from fumigant application until planting (Fig 4.3). The average high soil temperature at 3 pm was 32°C at 5 cm and 23°C at 20 cm. The average low temperatures at 8 am were 16°C at 5 cm and 19°C at 20 cm. An error was made during application of the 374 L/ha rate under TIF, therefore it was not included in the analysis. There were significant differences in retention between treatments (Fig 4.4). The 468 and 281 L/ha rates under TIF resulted in the greatest fumigant vapor concentrations within the bed, which were significantly greater than all other treatments through

nearly all sampling dates. The 187 L/ha rate under TIF resulted in the least fumigant concentrations; however these were often similar to the 468 and 561 L/ha rates under VIF. DMDS applied at 281 L/ha under TIF maintained greater fumigant concentration in the bed compared to the 468 and 561 L/ha rate under VIF for 28 DAF. Lowering fumigant rates to 187 L/ha under TIF provided similar or decreased DMDS concentrations compared to the standard 468 L/ha rate under VIF. The plant back period for the average low soil temperature at 20 cm was 28 DAF for VIF (Table 4.1). Fumigant concentrations at 28 DAF were low for the VIF treatments (18 - 25 ppm DMDS) and the 187 L/ha TIF treatment (11 ppm DMDS). DMDS concentrations remained above 25 ppm in other TIF treatments until 30 DAF for the 281 L/ha rate and 34 DAF for the 468 L/ha rate.

Fall 2010

Soil temperatures ranged from 19 to 41°C during the period from fumigation until planting (Fig 4.5). The average low soil temperature at 8 am was 25°C at 5 cm and 27°C at 20 cm. Average high soil temperatures at 3 pm were 38°C at 5 cm and 32°C at 20 cm. The standard application rate (468 L/ha) under TIF resulted in statistically greater fumigant concentrations compared to other treatments for most of the sampling period (Fig 4.6). Retention under VIF was similar for 468 and 561 L/ha rates. The retention of DMDS under VIF at both rates (468 and 561 L/ha) was generally similar to the lower fumigant rates (187 and 281 L/ha) under TIF while significantly lesser than the 374 L/ha rate under TIF. At the labeled plant back period for VIF films (21 DAF), the fumigant concentrations under VIF treatments were 25 – 33 ppm DMDS, and the DMDS concentration under TIF at the lowest application rate was 2 ppm DMDS (Table 4.1). Fumigant concentrations were below 25 ppm DMDS at 24 DAF for the 281 L/ha rate under TIF, 26 DAF for the 374 L/ha rate under TIF, 28 DAF for the 468 L/ha rate under TIF and 24 DAF for the 561 L/ha rate under VIF.

Spring 2011

Soil temperatures ranged from 13 to 49°C during the period after fumigation until fumigant planting (Fig 4.7). The mean low temperature at 8 am under TIF mulch at a depth of 5 cm was 21°C and at a depth of 20 cm it was 22°C. Average high soil temperatures at 3 pm were 36°C at 5 cm and 26°C at 20 cm. The standard DMDS application rate of 468 L/ha under VIF mulch was retained at similar concentrations as the higher rate of 561 L/ha under VIF and 281 L/ha under TIF (Fig 4.8). The standard rate (468 L/ha) under VIF was retained at a greater concentration than the 187 L/ha application rate under TIF until 25 days after fumigation. The reduced rate of 374 L/ha under TIF was retained at a greater concentration than the standard rate (468 L/ha) under VIF until 23 DAF. The standard rate (468 L/ha) under TIF was retained at the greatest concentration and was similar to the 374 L/ha rate under TIF. The labeled plant back period under VIF film at the observed mean low soil temperatures at 20 cm was 21 DAF (Table 4.1). The plant back period is 28 DAF for soil temperatures between 16 and 21.1°C and 21 DAF for soil temperatures 21.7°C and higher. The mean low temperature of 22°C at 20 cm was very close to the threshold between the two plant back intervals. Therefore, DMDS concentrations were greater than normal at the labeled plant back period. Measured DMDS was 1 ppm under TIF at an application rate of 187 L/ha and 58 – 73 ppm under VIF film for both application rates at 21 DAF. Fumigant concentrations were below 25 ppm DMDS at 23 DAF for 468 and 561 L/ha application rates under VIF, 25 DAF for 281 L/ha application rate under TIF, 28 DAF for 374 L/ha under TIF, and 37 DAF for 468 L/ha under TIF.

Fall 2011

Soil temperatures ranged from 21 to 39°C during the fumigant retention measurement period (Fig 4.9). Temperature at 8 am averaged 25°C at 5 cm and 26°C at 20 cm. Mean high soil temperature at 3 pm was 33°C at 5 cm and 28°C at 20 cm. The standard rate (468 L/ha) under VIF was retained at the least concentration until 13 days after fumigation and was retained at a lesser concentration than all the treatments under TIF until 17 days after treatment (Fig 4.10). The treatments under VIF were retained at similar concentrations regardless of application rate after 15 DAF. The lowest rate (187 L/ha) under TIF was retained at similar concentrations as the highest rate (468 L/ha) under TIF until 22 DAF. The labeled plant back period for DMDS under VIF was 21 DAF at the recorded mean low soil temps at 20 cm (Table 4.1). It remains doubtful if tomato could have been planted safely at 21 DAF for any treatment because DMDS concentrations remained high (30 – 2675 ppm). DMDS concentrations were below 25 ppm at 24 DAF for both VIF treatments, 29 DAF for 187 L/ha under TIF, 34 DAF for 281 L/ha, 31 DAF for 374 L/ha under TIF and 36 DAF for 468 L/ha under TIF.

Discussion

The EVOH barrier layer in TIF mulch is more retentive to DMDS than the polyamide (nylon) barrier layer in VIF. At an equal fumigant rate TIF always retained DMDS at greater concentrations for a longer period of time. Numerous studies have shown that VIF and TIF are significantly less permeable to fumigants than LDPE and HDPE (Gamliel et al., 1998a; Gamliel et al., 1998b; Gamliel et al., 1997; Wang et al., 1998; Yates et al., 2002; Ou et al., 2007; Santos et al., 2007; Wang et al., 1997; Chellemi et al., 2011; Gao et al., 2011a; Gao et al., 2011b; Qin et al., 2011; Fennimore and Ajwa, 2011).

The retention period was longer when soil temperatures were lower and during seasons with heavy rainfall. Various studies have found that film permeability, fumigant emissions and fumigant degradation decrease with decreasing temperatures (Papiernik and Yates, 2002; Wang et al., 1998; Gan et al., 1999; Ma et al., 2001; Guo and Gao, 2009). During fall 2011, heavy rainfall (26 cm in June according to the 2011 temperature and rainfall data from the ESAREC in Painter, VA) may have accounted for such high retention levels and long plant-back periods. According to the Paladin label the plant-back interval for DMDS is lengthened by heavy soils, low soil temperatures and high soil moisture. The planting interval post fumigation has been mandated by the registrant and its duration is based on average low soil temperature at a depth of 20 cm (Anonymous, 2012). Based on the registrant issued label, planting interval would have been 21 days during all fall seasons and in the spring of 2011 and 28 days during the spring of 2010 for VIF film. The concentration of fumigant vapor in the soil that can be tolerated by vegetable transplants is unclear but these data indicate that extensions in the planting interval may need to be implemented when applying DMDS under TIF mulch.

Conclusions

These data suggest that it may be possible to decrease DMDS application rates by 40 - 50% under TIF compared to VIF, while maintaining equal or greater fumigant vapor concentration within the bed for a similar or longer period of time. The 281 L/ha rate under TIF was retained similarly or at a greater concentration than the 468 and 561 L/ha rates under VIF. Buffer zones for DMDS applications can be reduced by TIF because application rates can be lowered. For example, a rate of 281 L/ha will reduce the buffer zone by 30 m compared to 468 L/ha for a 6 ha field. This is important because growers will be able to fumigate larger areas at a time, rather than fumigating numerous small blocks. In addition, growers can utilize larger

portions of their field for growing tomato, instead of leaving it out of production, especially if the field is near roads or residential areas. Under normal rainfall conditions the DMDS concentration was ≤ 25 ppm at the labeled plant back interval at the standard rate (468 L/ha) under VIF. The plant back interval may need to be increased by 2 to 7 days for reduced rates (281 and 374 L/ha) under TIF to reach similar fumigant concentrations as labeled plant-back periods under VIF. The fumigant concentrations in this study were not always significantly greater under TIF mulch at the planting interval time but may be biologically significant and may cause phytotoxicity.

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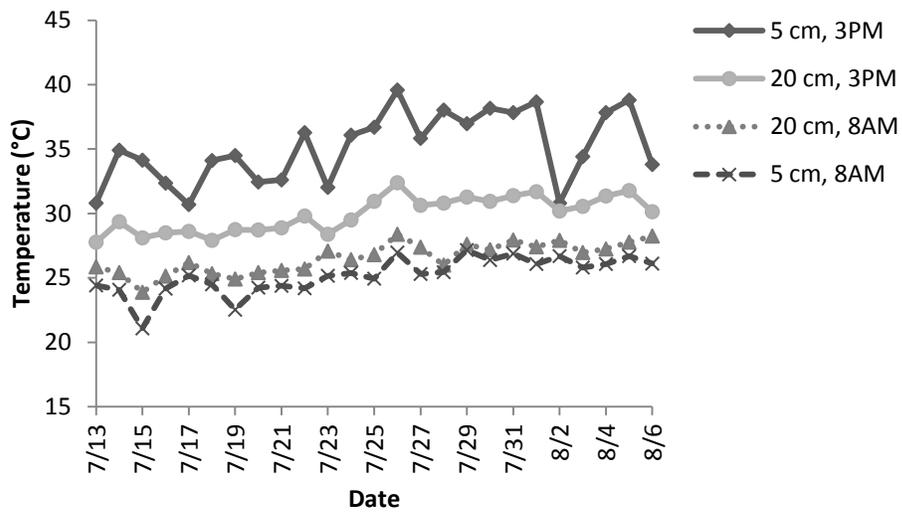


Fig. 4.1 Soil temperatures at 5 cm and 20 cm depths at 8 am and 3 pm under totally impermeable film (TIF) mulch in a shank applied dimethyl disulfide (DMDS) experiment. Experiments were performed during fall 2009 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA.

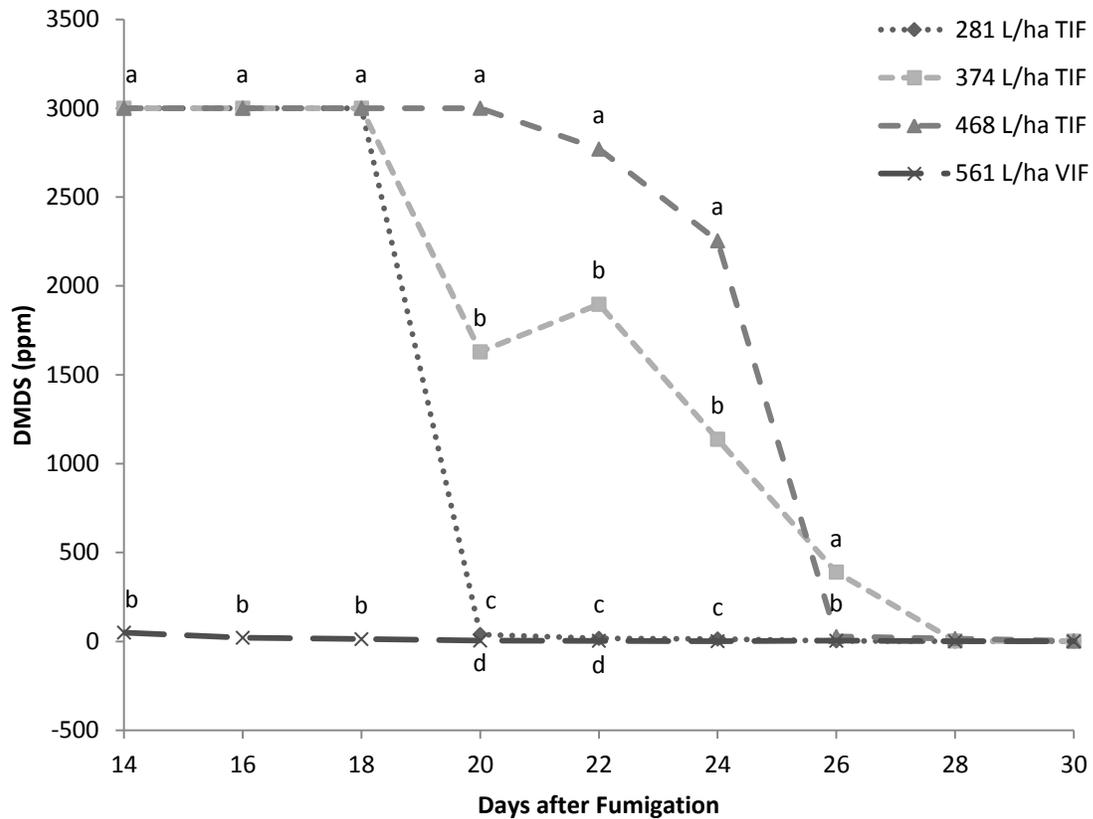


Fig. 4.2. Retention of shank applied dimethyl disulfide (DMDS) under virtually impermeable film (VIF) and totally impermeable film (TIF) mulches. Experiments were performed during fall 2009 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA. Means followed by the same letter are not significantly different at $P \leq 0.05$ by Duncan's multiple range test. Means are compared within the same day.

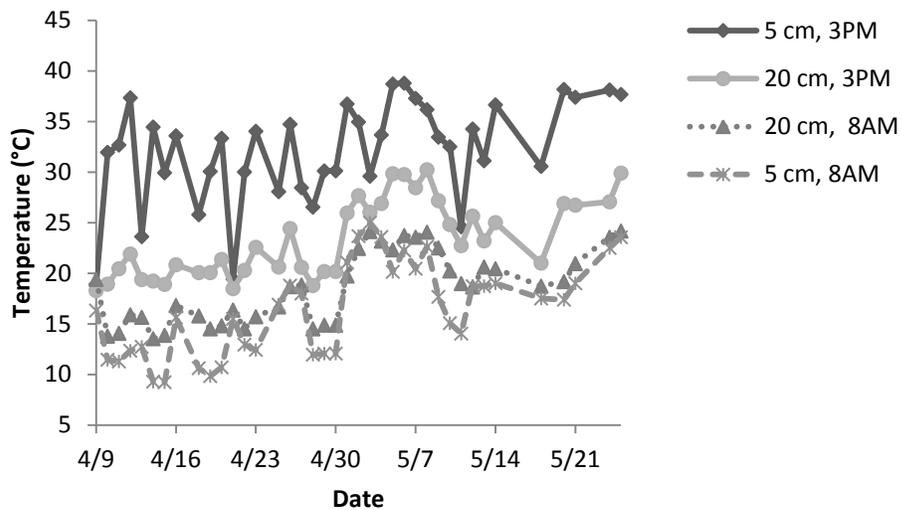


Fig. 4.3. Soil temperatures at 5 cm and 20 cm depths at 8 am and 3 pm under totally impermeable film (TIF) mulch in a shank applied dimethyl disulfide (DMDS) experiment. Experiments were performed during spring 2010 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA.

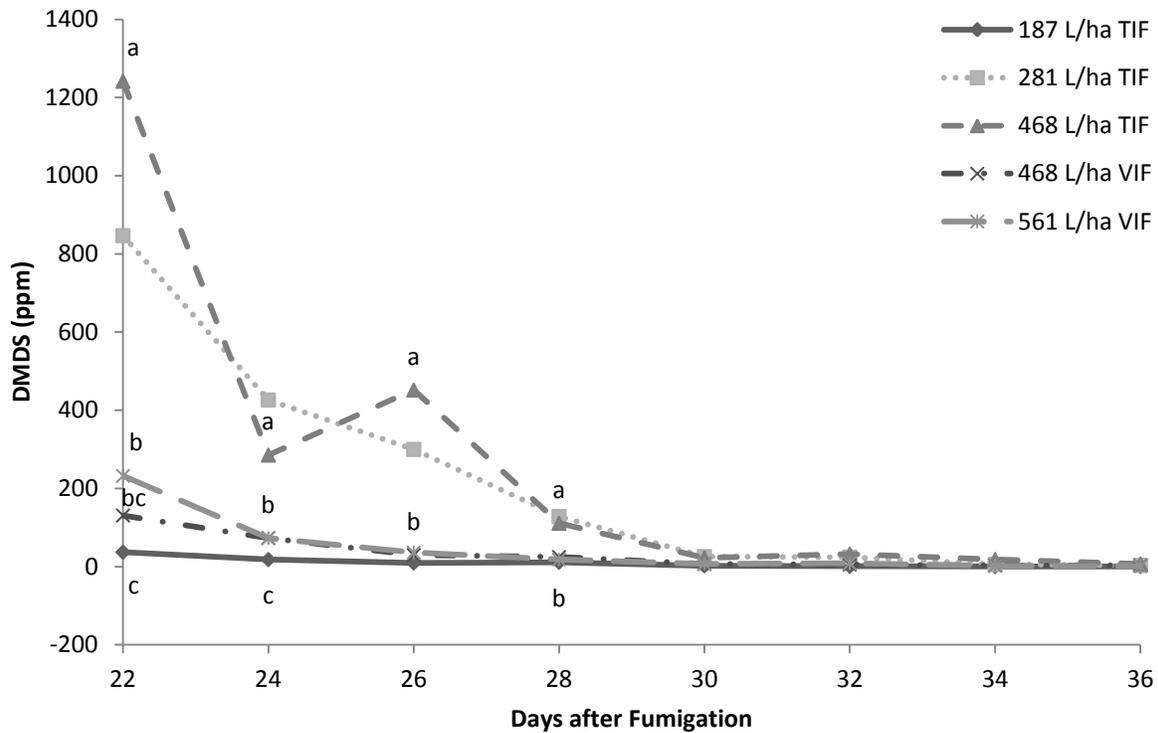


Fig. 4.4. Retention of shank applied dimethyl disulfide (DMDS) under virtually impermeable film (VIF) and totally impermeable film (TIF) mulches. Experiments were performed during spring 2010 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA. Means followed by the same letter are not significantly different at $P \leq 0.05$ by Duncan's multiple range test. Means are compared within the same day.

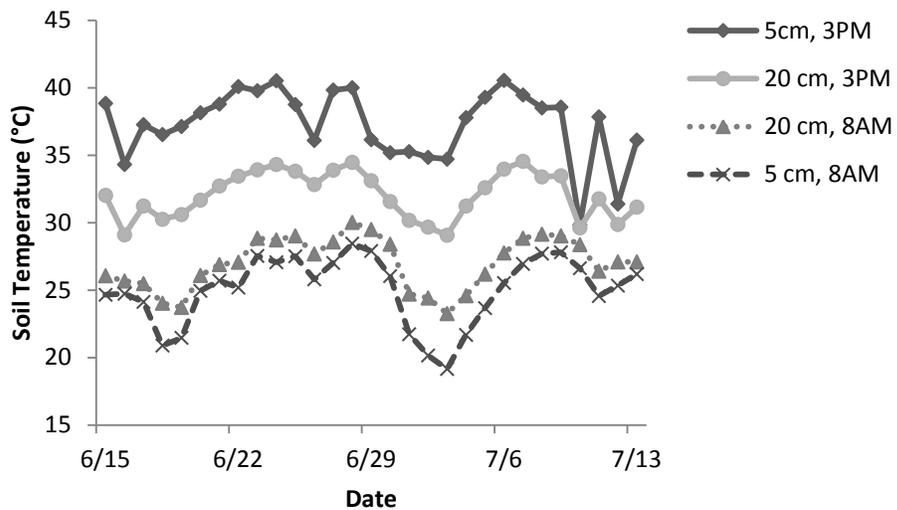


Fig. 4.5. Soil temperatures at 5 cm and 20 cm depths at 8 am and 3 pm under totally impermeable (TIF) mulch in a shank applied dimethyl disulfide (DMDS) experiment. Experiments were performed during fall 2010 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA.

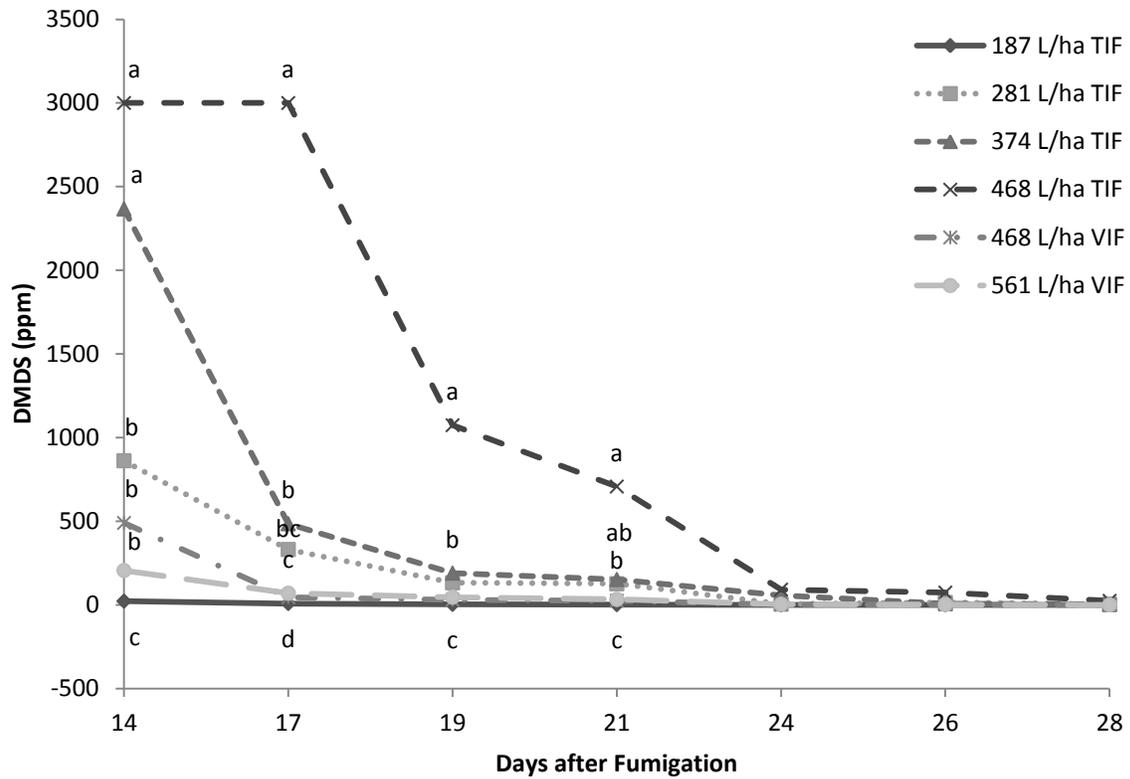


Fig. 4.6. Retention of shank applied dimethyl disulfide (DMDS) under virtually impermeable film (VIF) and totally impermeable film (TIF) mulches. Experiments were performed during fall 2010 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA. Means followed by the same letter are not significantly different at $P \leq 0.05$ by Duncan's multiple range test. Means are compared within the same day.

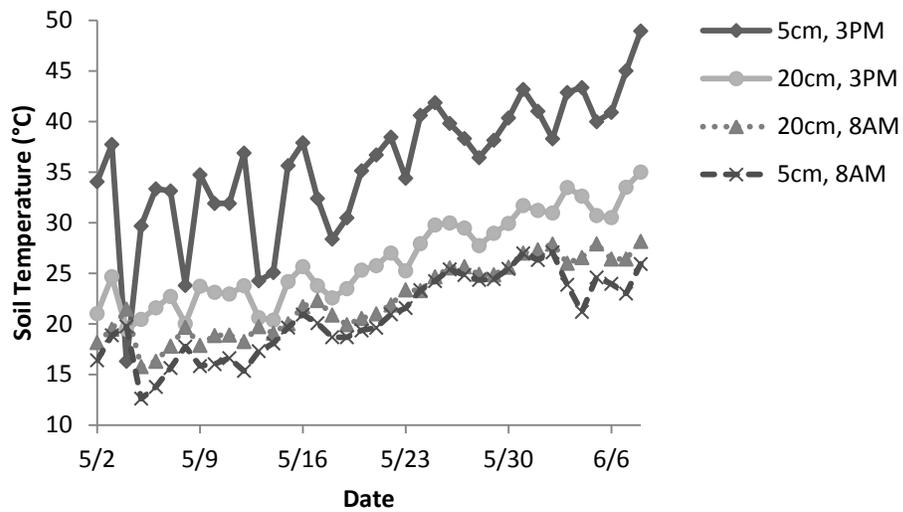


Fig. 4.7. Soil temperatures at 5 cm and 20 cm depths at 8am and 3pm under totally impermeable film (TIF) mulch in a shank applied dimethyl disulfide (DMDS) experiment. Experiments were performed during spring 2011 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA.

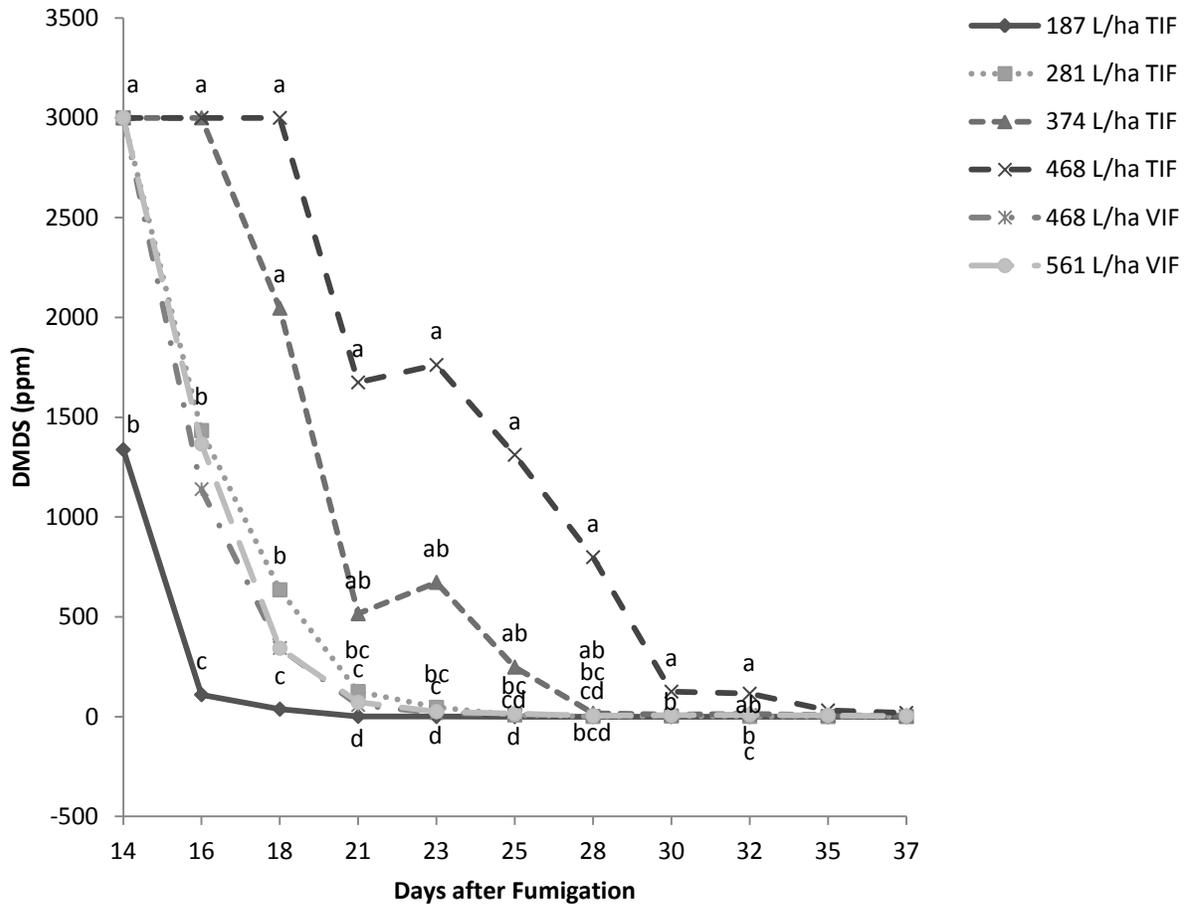


Fig. 4.8. Retention of shank applied dimethyl disulfide (DMDS) under virtually impermeable film (VIF) and totally impermeable film (TIF) mulches. Experiments were performed during spring 2011 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA. Means followed by the same letter are not significantly different at $P \leq 0.05$ by Duncan's multiple range test. Means are compared within the same day.

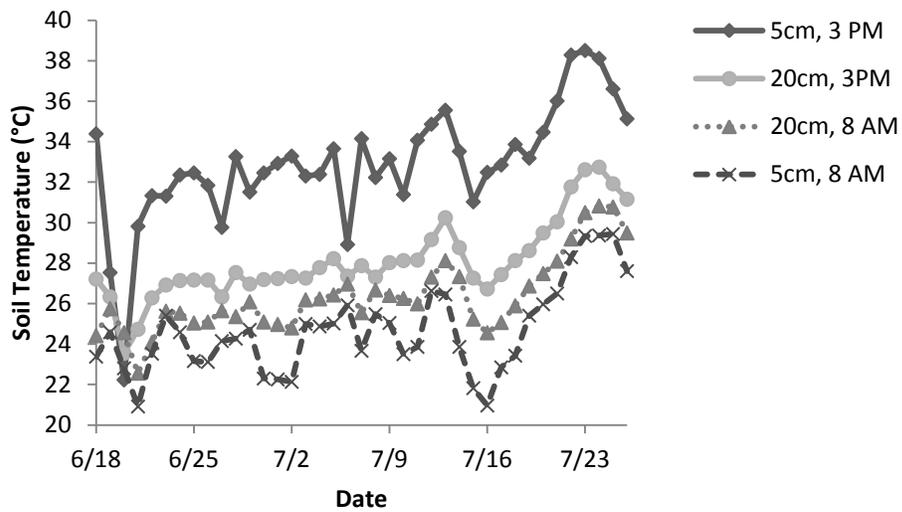


Fig. 4.9. Soil temperatures at 5 cm and 20 cm depths at 8 am and 3 pm under totally impermeable film (TIF) mulch in shank applied dimethyl disulfide (DMDS) experiment. Experiments were performed during fall 2011 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA.

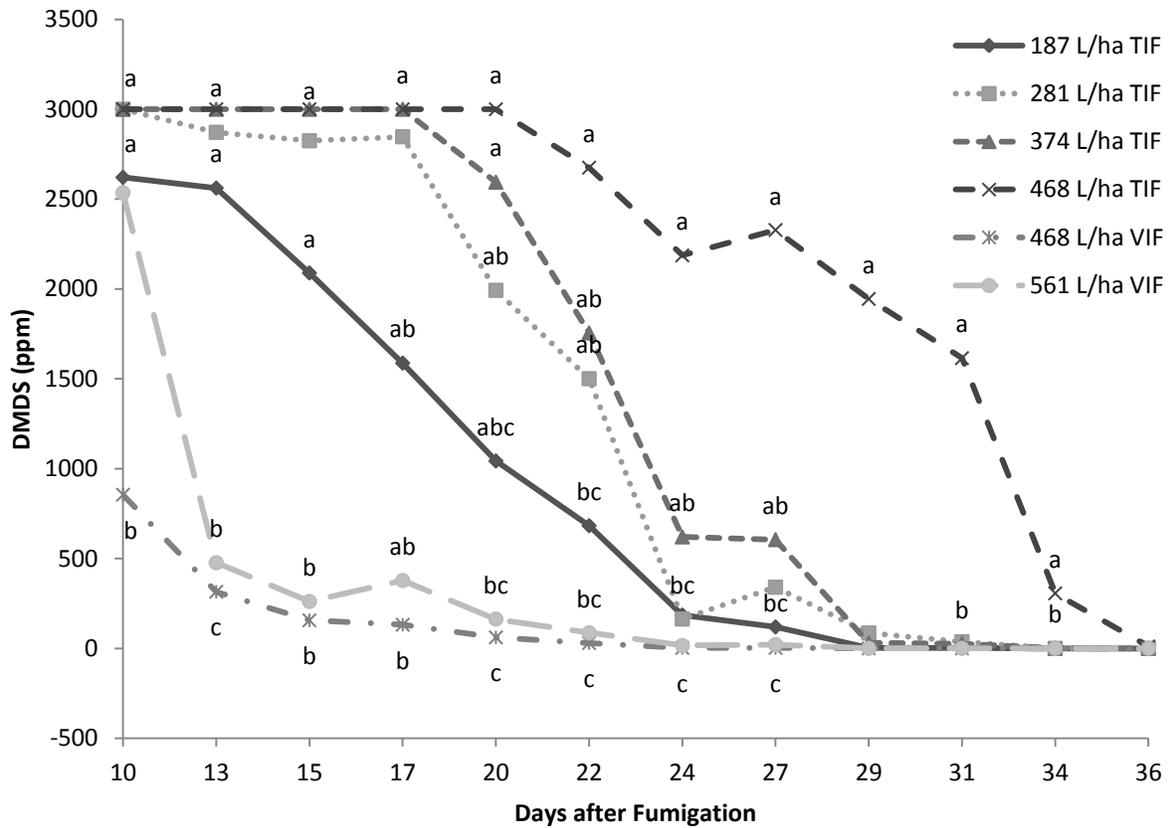


Fig. 4.10. Retention of shank applied dimethyl disulfide (DMDS) under virtually impermeable film (VIF) and totally impermeable film (TIF) mulches. Experiments were performed during fall 2011 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA. Means followed by the same letter are not significantly different at $P \leq 0.05$ by Duncan's multiple range test. Means are compared within the same day.

Table 4.1 Dimethyl disulfide (DMDS) concentrations (ppm) under virtually impermeable film (VIF) and totally impermeable film (TIF) mulch at labeled planting intervals in shank applied DMDS experiments. Labeled planting intervals are expressed as days after fumigation (DAF). Plant back intervals are based upon average low soil temperatures at 20 cm when used with approved labeled VIF and metalized mulches. DMDS is not currently labeled for use with Vaporsafe TIF. DAF values for DMDS concentrations below 25 ppm are indicated.

Season	Treatment	Labeled Plant Back Interval (DAF)	DMDS concentration (ppm) at plant back	DAF required to reach DMDS concentration below 25 ppm
Fall 2009	281 L/ha TIF	21	39	22
	374 L/ha TIF	21	1629	28
	468 L/ha TIF	21	3000	28
	561 L/ha VIF	21	5	16
Spring 2010	187 L/ha TIF	28	11	24
	281 L/ha TIF	28	128	30
	468 L/ha TIF	28	111	34
	468 L/ha VIF	28	25	28
	561 L/ha VIF	28	18	28
Fall 2010	187 L/ha TIF	21	2	14
	281 L/ha TIF	21	126	24
	374 L/ha TIF	21	152	26
	468 L/ha TIF	21	708	28
	468 L/ha VIF	21	25	21
	561 L/ha VIF	21	33	24
Spring 2011	187 L/ha TIF	21	1	21
	281 L/ha TIF	21	126	25
	374 L/ha TIF	21	516	28
	468 L/ha TIF	21	1674	37
	468 L/ha VIF	21	58	23
	561 L/ha VIF	21	73	23
Fall 2011	187 L/ha TIF	21	683	29
	281 L/ha TIF	21	1501	34
	374 L/ha TIF	21	1755	31
	468 L/ha TIF	21	2675	36
	468 L/ha VIF	21	30	24
	561 L/ha VIF	21	87	24

Chapter 5 Retention of Methyl Iodide under Virtually and Totally Impermeable Films

Introduction

Methyl iodide (MeI), also referred to as iodomethane, is an alternative to methyl bromide (MBr) for pre-plant soil fumigation (Duniway, 2002). Title 5, section 602 of the Clean Air act orders the U.S. EPA to list any substance with an “Ozone Depletion Potential” (ODP) of 0.2 or greater as a Class 1 ozone depleter. The ODP of methyl iodide is likely less than 0.016, which is much lower than the level of Class 1 ozone depleters (Ohr et al., 1996). In nature, MeI is produced by marine algae and is uniformly distributed in the ocean. The gas is a general biocide like methyl bromide. MeI is the most effective fumigant compared to other alkyl iodides. MeI is a better methylating agent than MBr and equal or better at controlling certain soilborne pathogens and weeds than MBr at equivalent molar rates (Ohr et al., 1996). MeI is an ozone safe alternative to MBr due to rapid degradation by UV light (Ohr et al., 1996). MeI does not persist long in the atmosphere having an atmospheric residence time of only 4 to 8 days compared with 2 years for methyl bromide. MeI is very costly compared to other soil fumigants, therefore reduced application rates would be beneficial to growers (Gilreath and Santos, 2011). The objective of this study was to test VIF and TIF on MeI retention at reduced application rates under field conditions during different seasons in Virginia.

Materials and Methods

MeI fumigant retention experiments were conducted at the Virginia Tech Eastern Shore Agricultural Research and Extension Center (ESAREC) in Painter, VA during the spring and fall of 2010 and 2011. Soil type at ESAREC is a Bojac sandy loam (Thermic Typic Hapludults) with 59% sand, 30% silt, and 11% clay with pH ranging from 6.2 to 6.5 and organic matter content of 0.50 to 0.75%. Soil was cultivated to a depth of 30 cm prior to fumigation. If necessary, overhead sprinkler irrigation was used to bring soil moisture capacity to between 50 and 75% field capacity. The fumigant formulation we used was MeI:chloropicrin (Pic) 50:50 w/w (Arysta LifeScience Corporation, Cary, NC, USA) applied using a single row combination bed press with bed dimensions 76 cm wide and 20 cm high with three back swept shanks. Shanks were 20 cm long and fumigant was released at the bottom of the shank.

The treatments in the experiment were a standard rate for highly retentive films (93.3 L/ha (178 kg/ha broadcast)) under VIF and TIF, and reduced rates (37.3 L/ha (71.2 kg/ha broadcast), 56 L/ha (106.8 kg/ha broadcast), 74.6 L/ha (142.5 kg/ha broadcast)) under TIF. Plots were arranged in a randomized complete block design with four replications. Experimental plots were 24 m long with a between row spacing of 1.8 m. Black films were used in the spring and white on black were used in the fall seasons. The mulch types used were 0.05 mm thick Vaporsafe® TIF (Raven Industries Inc., Sioux Falls, SD, USA), which utilized an EVOH barrier layer and 0.03 mm thick embossed Pliant Blockade® VIF (Berry Plastics Corp., Evansville, IN, USA), which utilized a nylon barrier layer. The labeled fumigant rate for states other than Florida under highly retentive tarps in tomato to control nutsedge is a minimum of 94.4 L/ha (179.2 kg/ha broadcast) and the plant-back period is 14 to 21 days. Fumigant application rates were adjusted by flow rate (measured by King® flow meter (King Instrument Company, Garden Grove, CA, USA) using a 10W float (0.75 L/min of water at 100% flow) and tractor speed. In order to achieve uniform fumigant delivery between chisels in the bed using low fumigant rates, a small diameter tubing (1.59 mm) was used, and lines were fully charged before fumigating

plots as described by Gilreath et al. (2005). Fumigants were applied on 15 April 2010, 18 June 2010, 27 April 2011, and 11 August 2011.

Data Collection

Soil temperature and fumigant persistence were collected for these experiments. Fumigant persistence was measured using a MiniRAE3000 volatile organic compound (VOC) meter (RAE Systems, San Jose, CA, USA). The VOC detector uses a lamp and an ultraviolet (UV) light to ionize compounds that can be counted by a photo ionization detector (PID) (Noling et al., 2008). A MiniRAE VOC meter was used to measure 1,3-D plus Pic retention under TIF in a similar study (Fennimore and Ajwa, 2011). We used a 9.8 electron volt (eV) ionization energy lamp which does not have a response to Pic, only MeI was detected by the lamp (Anonymous 2012). The maximum readable concentration for the MiniRAE 3000 is 3,150 ppm MeI. A particle filter was attached to the VOC meter probe to prevent soil particles and moisture from entering the meter. VOC readings were taken every other day after the fumigants started to dissipate until planting. VOC sampling was performed during the afternoon on sampling dates. Samples were taken equidistant between the bed center and the bed shoulder. A 1.3 cm diameter round wooden dowel was inserted vertically to a depth of 10 cm. The dowel was removed and the probe was immediately inserted into the headspace. A seal was formed between the mulch and particle filter and readings continued until values stabilized or decreased and maximum values were recorded. Three sub-samples were taken from each plot and averaged. Sampling holes were covered with Poly Tarp Tape (BAC Industries Inc., Minneapolis, MN, USA) after measurement to prevent gas escape. Subsequent samples were taken from separate sampling holes.

Soil temperatures under the plastic were measured using two S-TMB-M002 12-bit Temperature/RH Smart Sensors (Onset Computer Corp., Bourne, MA, USA). These sensors were attached to a HOBO micro station data logger (Onset Computer Corp., Bourne, MA, USA), which recorded and stored the data. Temperatures were measured within the fumigated zone at depths of 5 cm and 20 cm. The data logger took measurements were taken every 30 seconds and averaged them every two minutes. Temperatures at both depths are reported for 8 am and 3 pm. These represent the daily maximum and minimum temperatures.

Data Analysis

The retention data was transformed using a log transformation. Statistical differences between transformed treatment means were determined by analysis of variance (ANOVA) by day after fumigation (DAF). Significant differences between treatment means were separated using Duncan's multiple range test at $P \leq 0.05$.

Results

Spring 2010

Soil temperatures ranged between 9 to 39°C from soil fumigation until planting (Fig 5.1). Average low soil temperature at 8 am was 16°C at 5 cm and 19°C at 20 cm. Mean high temperature at 3 pm was 32°C at 5 cm and 23°C at 20 cm. The standard rate (93.3 L/ha) under TIF was retained at the greatest concentrations compared to the other treatments except for the 74.6 L/ha under TIF treatment until 13 days after fumigation (DAF) and the 93.3 L/ha under VIF treatment until 11 DAF (Fig 5.2). The standard rate (93.3 L/ha) under VIF was retained similarly to the reduced 56 L/ha and 74.6 L/ha rates under TIF treatments throughout the experiment. The lowest rate (37.3 L/ha) under TIF was retained at a lesser concentration than the other treatments until 13 DAF. All treatments had negligible soil fumigant concentrations at

the labeled plant back interval. The labeled plant back interval is 14-21 DAF when using a standard rate (93.3 L/ha to control nutsedge) under approved highly retentive tarps.

Fall 2010

Soil temperatures ranged from 21 to 41°C (Fig 5.3). Mean low soil temperature at 8 am was 26°C at 5 cm and 28 at 20 cm. Average high soil temperature at 3 pm was 38°C at 5 cm and 33°C at 20 cm. The standard rate under TIF was retained the greatest concentration and retention levels were similar to the 74.6 L/ha under TIF treatment at 6 DAF (Fig 5.4). The standard rate under VIF was retained at similar concentrations as the 37.3 L/ha and 56 L/ha treatments under TIF. The reduced rate of 74.6 L/ha under TIF was retained at a greater level than the standard rate (93.3 L/ha) under VIF. Safe plant back period was achieved by 10 DAF for all treatments, which is less than the labeled plant back period.

Spring 2011

Soil temperatures ranged from 13 to 43°C (Fig 5.5). Average low soil temperature at 8 am was 23°C 5 cm and 25°C at 20 cm. Mean high soil temperature at 3 pm was 33°C at 5 cm and 27°C at 20 cm. Midas retention was not different between treatments at 2 DAF (Fig 5.6). At 5 and 8 DAF all treatments retained MeI at similar concentrations, except for the highly reduced 37.3 L/ha rate under TIF which had lesser concentrations compared to the other treatments. After 9 DAF there were no differences in MeI retention between treatments. At the labeled plant back period, fumigant had dissipated from all treatments.

Fall 2011

Soil temperatures ranged from 23 to 36°C (Fig 5.7). Average low soil temperature at 8 am was 24°C at 5 cm and 27°C at 20 cm. Mean high soil temperature at 3 pm was 31°C at 5 cm and 28°C at 20 cm. Initially there were no differences in MeI retention between treatments (Fig 5.8). 93.3 L/ha, 74.6 L/ha, and 56 L/ha under TIF treatments had similar retention concentrations 4 DAF. The standard rate under VIF was retained at similar concentrations as the 37.3 L/ha rate under TIF at 4 DAF. The standard rate under TIF was retained at the greatest concentration, while the other treatments maintained similar MeI concentrations at 7 and 9 DAF. All the treatments could be safely planted during the labeled plant back period.

Discussion

TIF can be implemented to increase MeI retention and decrease fumigation rates compared to VIF. Numerous studies have shown that VIF and TIF are significantly less permeable to fumigants than LDPE and HDPE (Gamliel et al., 1998a; Gamliel et al., 1998b; Gamliel et al., 1997; Wang et al., 1998; Yates et al., 2002; Ou et al., 2007; Santos et al., 2007; Wang et al., 1997; Chellemi et al., 2011; Gao et al., 2011a; Gao et al., 2011b; Qin et al., 2011; Fennimore and Ajwa, 2011). Compared to other fumigants MeI seems to quickly dissipate under TIF and it appears plant back periods do not need to be extended. During the first two seasons MeI concentrations had rapidly dissipated by the time VOC readings were initiated. VOC readings were subsequently started at 2 days after fumigation before the re-entry period had terminated while wearing a respirator to obtain a better understating of MeI concentrations under highly impermeable films. MeI retention periods were longer in the spring when soil temperatures were cooler, and shorter in the fall when soil temperatures were warmer. Various studies have found that film permeability, fumigant emissions and fumigant degradation increase with increasing temperatures (Papiernik and Yates, 2002; Wang et al., 1998; Gan et al., 1999; Ma et al., 2001; Guo and Gao, 2009).

Conclusions

MeI rates could be safely reduced by 40% (from 93.3 to 56 L/ha) when using TIF compared to VIF while maintaining similar or greater MeI concentrations under the plastic film. Based upon our results the labeled plant back periods are adequate during both the spring and fall seasons under both the VIF and TIF for the various rates tested. Therefore, reduced MeI rates under TIF can be used while maintaining fumigant concentration and not increasing plant back intervals. Buffer zone distances may be lowered due to lowered application rates and greater retention levels (less emission into atmosphere). For example the buffer zone could be reduced from 42 m to 25 m if the application rate was lowered from 93.3 L/ha to 56 L/ha on an 8 ha field.

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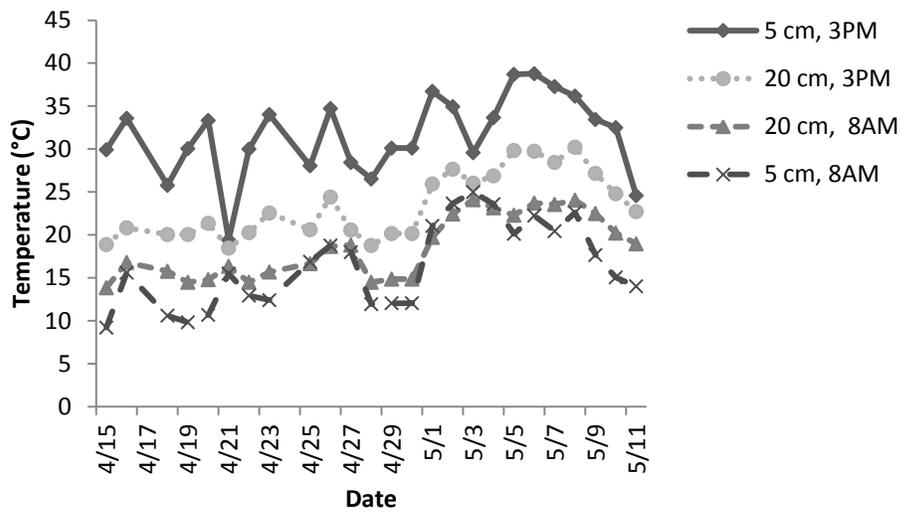


Fig. 5.1. Soil temperatures at 5 cm and 20 cm depths at 8 am and 3 pm under totally impermeable film (TIF) mulch in a methyl iodide (MeI) experiment. Experiments were performed during spring 2010 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA.

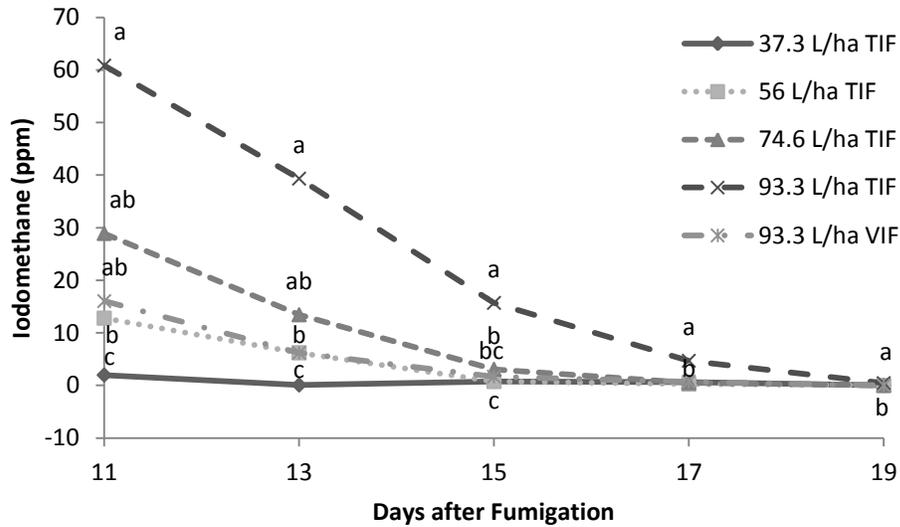


Fig. 5.2. Retention of methyl iodide (MeI) under virtually impermeable film (VIF) and totally impermeable film (TIF) mulches. Experiments were performed during spring 2010 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA. Means followed by the same letter are not significantly different at $P \leq 0.05$ by Duncan's multiple range test. Means are compared within the same day.

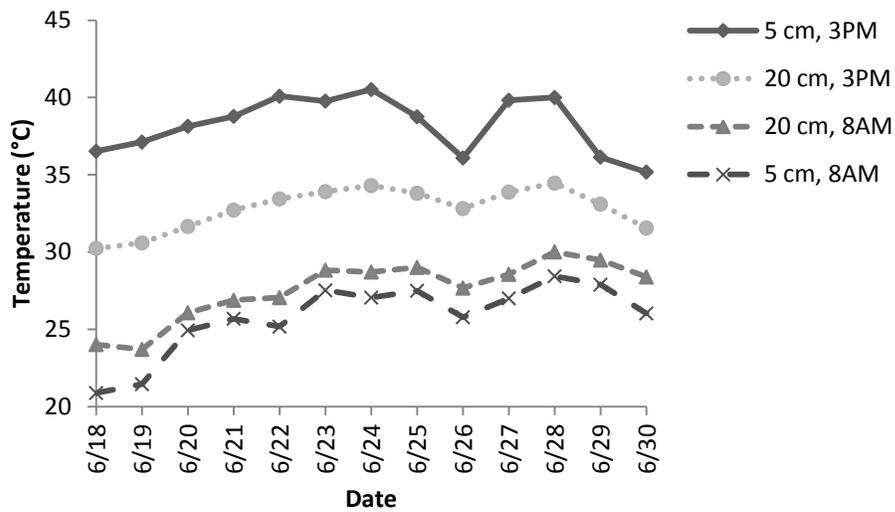


Fig. 5.3 Soil temperatures at 5 cm and 20 cm depths at 8 am and 3 pm under totally impermeable film (TIF) mulch in a methyl iodide (MeI) experiment. Experiments were performed during fall 2010 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA.

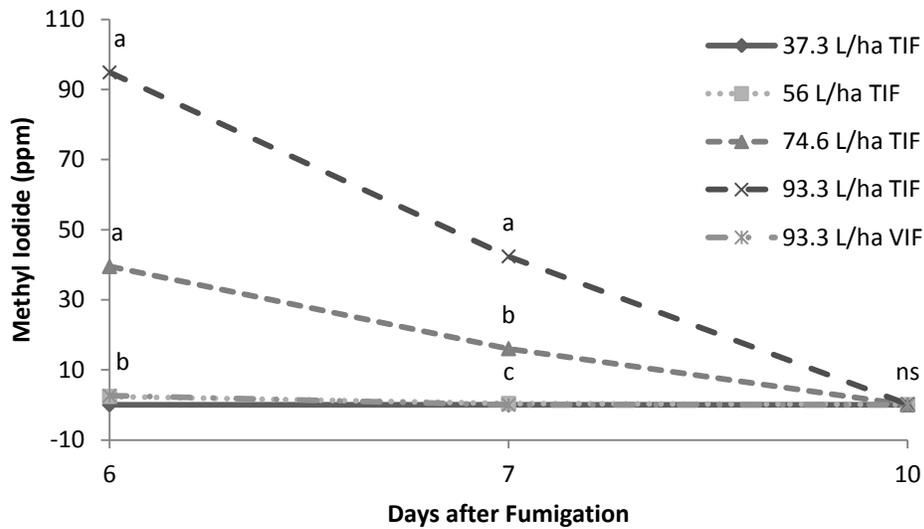


Fig. 5.4. Retention of methyl iodide (MeI) under virtually impermeable film (VIF) and totally impermeable film (TIF) mulches. Experiments were performed during fall 2010 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA. Means followed by the same letter are not significantly different at $P \leq 0.05$ by Duncan's multiple range test. Means are compared within the same day.

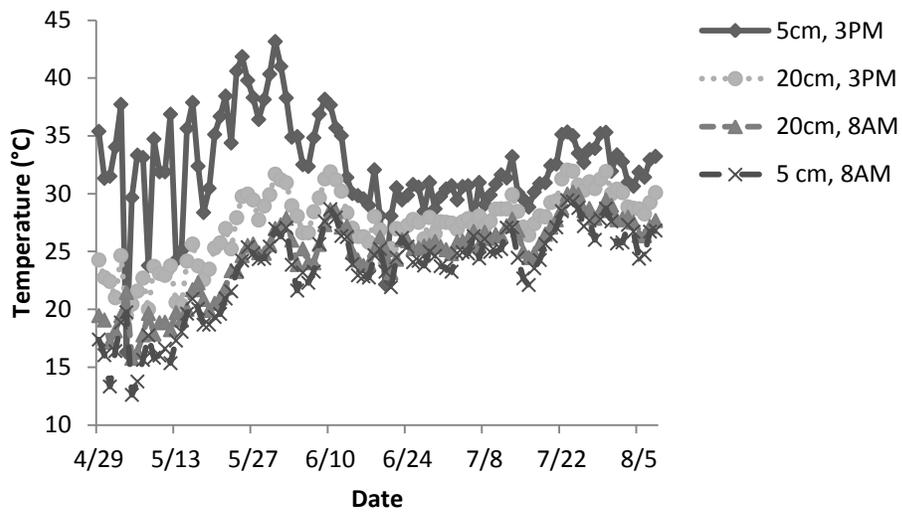


Fig. 5.5. Soil temperatures at 5 cm and 20 cm depths at 8 am and 3 pm under totally impermeable film (TIF) mulch in a methyl iodide (MeI) experiment. Experiments were performed during spring 2011 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA.

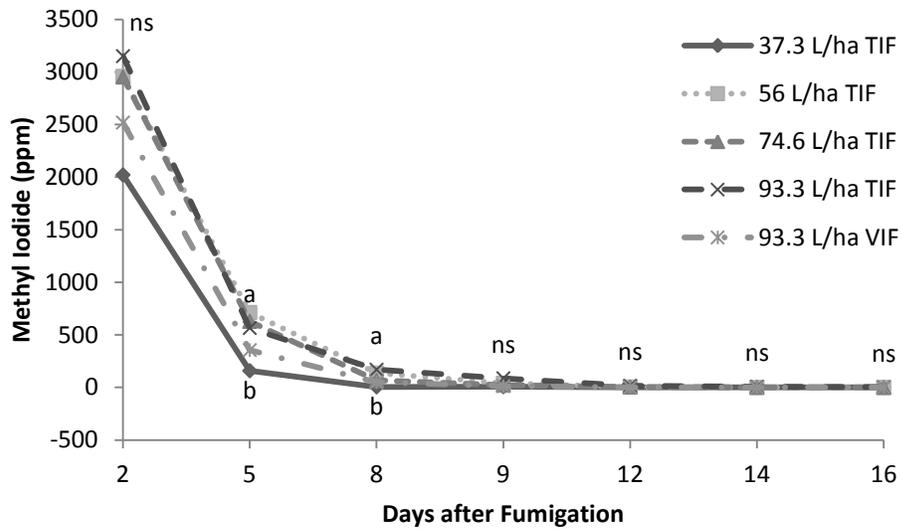


Fig. 5.6 Retention of methyl iodide (MeI) under virtually impermeable film (VIF) and totally impermeable film (TIF) mulches. Experiments were performed during spring 2011 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA. Means followed by the same letter are not significantly different at $P \leq 0.05$ by Duncan's multiple range test. Means are compared within the same day.

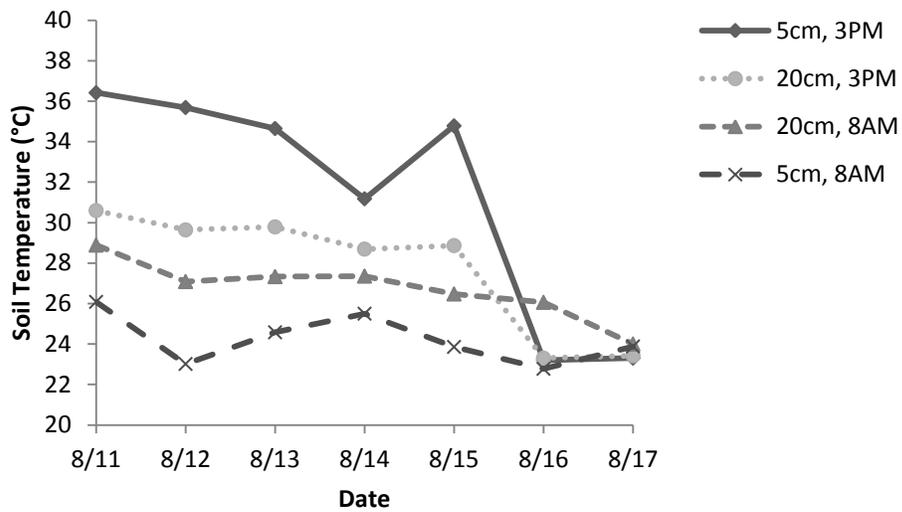


Fig. 5.7. Soil temperatures at 5 cm and 20 cm depths at 8am and 3pm under totally impermeable film (TIF) mulch in a methyl iodide (MeI) experiment. Experiments were performed during fall 2011 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA.

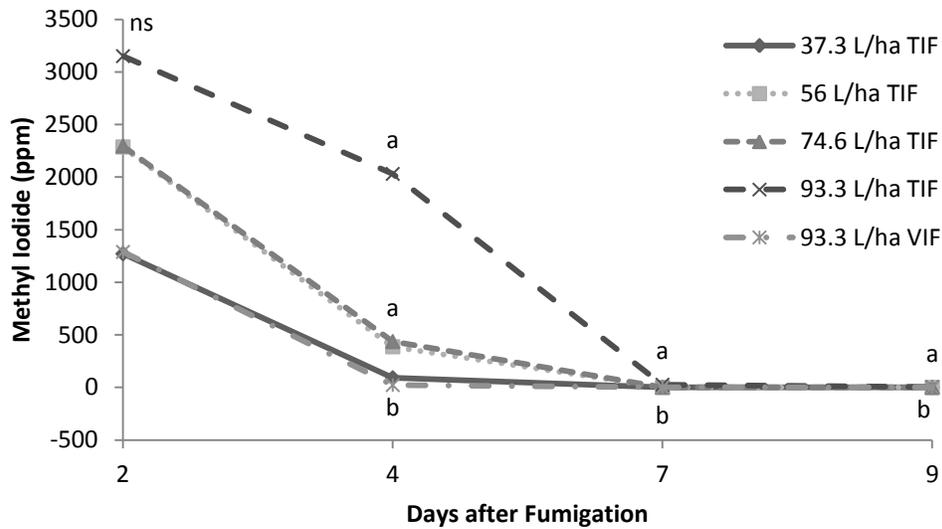


Fig. 5.8. Retention of methyl iodide (MeI) under virtually impermeable film (VIF) and totally impermeable film (TIF) mulches.. Experiments were performed during fall 2011 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA. Means followed by the same letter are not significantly different at $P \leq 0.05$ by Duncan's multiple range test. Means are compared within the same day.

Chapter 6 Retention of Drip Applied Dimethyl Disulfide by Virtually and Totally Impermeable Films

Introduction

Chemigation is the process of applying chemicals to the soil or plant through irrigation water (Ajwa et al., 2002). Applying chemicals through irrigation may provide more uniform distribution without disturbing or compacting the soil. Drip fumigation under high density polyethylene (HDPE) film resulted in more uniform distribution of 1,3-dichloropropene (1,3-D) in soil compared to a shank injected application (Ajwa et al., 2002). In addition, the process requires little extra equipment and energy. Emulsifiable concentrate (EC) fumigant formulations can be applied through the same irrigation systems that are subsequently used to water the crop. Fumigant application through drip irrigation can be economical, and likely reduce emissions, worker exposure, and application rates compared to conventional shank application (Ajwa et al., 2002). Ou et al. (2005) suggest that drip fumigants should be more effective than shank delivered fumigants, because the chemical will be dissolved in the water phase and directly contact soil microorganisms, which are coated in a layer of water. The objective of this study was to evaluate the retention of drip applied dimethyl disulfide (DMDS) under TIF and VIF for multiple seasons in Virginia.

Materials and Methods

Experiments to compare the retention of drip and shank applied DMDS under VIF and TIF were conducted at the Virginia Tech Eastern Shore Agricultural Research and Extension Center (ESAREC) in Painter, VA during the fall of 2009, spring and fall of 2010, and spring and fall of 2011. Soil type at ESAREC is a Bojac sandy loam (Thermic Typic Hapludults) with 59% sand, 30% silt, and 11% clay with pH ranging from 6.2 to 6.5 and organic matter content of 0.50 to 0.75%. Soil was cultivated to a depth of 30 cm prior for bed preparation. If necessary, overhead sprinkler irrigation was used to bring soil moisture capacity to between 50 and 75% field capacity. Experimental plots were 24 m long with a between row spacing of 1.8 m. Experimental plots were arranged as a randomized complete block design with four replications. The experiment included three drip applied treatments (374 L/ha (428 kg/ha broadcast) and 468 L/ha (535 kg/ha broadcast) under TIF and 561 L/ha (642 kg/ha broadcast) under VIF) and one shank treatment (468 L/ha (535 kg/ha broadcast)). The fall 2009 experiment did not include the drip applied 374 L/ha under TIF treatment. The shank treatment used a 79:21 w/w formulation of DMDS:chloropicrin (Pic) (United Phosphorus Inc., King of Prussia, PA, USA) that was applied using a single-row combination bed press 76 cm wide and 20 cm high with three back swept shanks. Shanks were 20 cm long and fumigant was released at the bottom of the shank. The drip applied treatments used a 79:21 w/w emulsifiable concentrate formulation of DMDS:Pic (United Phosphorus Inc., King of Prussia, PA, USA). The shank applied fumigant rate was achieved with a Siemens® (Siemens Corporation, New York, NY, USA) flow meter using a float (float code 708) rated for 3.5 L/min of DMDS:Pic 79:21 at 100% flow and constricting the flow with a TeeJet® (TeeJet Technologies, Wheaton, IL, USA) flow regulator (orifice plates) as described by Gilreath et al. (2005). Bed dimensions were similar between drip and shank treatments. However, the drip treatments contained two drip tapes while the shank treatments contained only one drip tape. Drip applied DMDS was delivered after yellow nutsedge sprouted and began to emerge through the plastic, generally 7 days after plastic deployment. Fumigants are more effective when weed germination is initiated and the sprouting

seeds or vegetative propagules are exposed to the chemical. Ou et al. (2005) suggest that drip fumigants should be more effective than shank delivered fumigants because the chemical will be dissolved in the water phase and directly contact soil microorganisms which are coated in a layer of water.

Drip applied DMDS was delivered from individual cylinders pressurized with carbon dioxide (similar to the nitrogen pressurized cylinder system described by Ajwa et al., 2002). Known quantities of DMDS were added into the stainless steel spray containers based upon weight. The emulsified fumigant was metered into the drip irrigation with pressure regulators through a manifold which contained a water flow rate regulator and a back-flow preventer. The manifold then branched into three separate lines so all the treatments could be delivered simultaneously. Fumigant delivery was monitored as weight loss over time (by using scales to weigh the cylinders) and application rate was adjusted by increasing or decreasing flow rate out of the cylinder. The fumigant was dispersed uniformly with 126,945 L/ha of water delivered by two equally spaced drip tapes (Aqua-Traxx® with the PBX advantage, The TORO Company, El Cajon, CA, USA) operating at a flow rate of 1.7 L/min/30 m over a three hour period. Desaegeer et al. (2004) found that drip applied 1,3-D:Pic (60.8:33.3 w/w) delivered through two drip tapes improved water movement and resulted in a more uniform fumigation pattern compared to a single drip tape. In addition, irrigation volumes greater than applied in this experiment resulted in greater lateral movement, but reduced fumigant activity. The system was flushed with water for the last 20 minutes after fumigant application. Beds were formed for all treatments and shank DMDS applications were made on 1 July, 2009, 23 April 2010, 17 June 2010, 26 April 2011, and 23 June 2011. Drip applied DMDS was delivered on 9 July 2009, 3 May 2010, 22 June 2010, 6 May 2011, and 11 July 2011. Drip applications were made 5 to 10 days after the mulch was laid to allow for the nutsedge tubers to sprout. In fall 2011, excessive rainfall (16 cm at the ESAREC from bed formation until drip application (ESAREC 2011)) delayed drip application until 18 days after the bed were formed and covered with plastic.

Two mulches were used to compare DMDS retention. During all experiments, either a black (spring) or white on black (fall) formulation of Blockade® VIF (Berry Plastics Corp., Evansville, IN, USA) embossed polyethylene mulch with thickness 0.03 mm containing a nylon barrier was used. Black (spring), or white on black (fall) Vaporsafe® TIF (Raven Industries Inc., Sioux Falls, SD, USA) polyethylene mulch with 0.05 mm thickness containing an EVOH barrier was used. A white on black formulation of TIF was not available during the fall of 2009, so the mulch was painted white the day after application with white exterior latex paint. Comparative temperature readings were taken between the painted mulch and factory white VIF mulch and found to be statistically similar (data not presented). The labeled rate for PaladinEC® under VIF film in tomatoes to control nutsedge is 593 L/ha (679 kg/ha broadcast) and 561 L/ha for shank applied Paladin. DMDS is not currently labeled for use with Vaporsafe® TIF.

Data Collection

Soil temperature and fumigant persistence were collected for these experiments. Fumigant persistence data was measured using a MiniRAE3000 volatile organic compound (VOC) meter (RAE Systems, San Jose, CA, USA). The VOC detector uses a lamp and an ultraviolet (UV) light to ionize compounds that can be counted by a photo ionization detector (PID) (Noling et al., 2008). A MiniRAE VOC meter was used to measure 1,3-D plus Pic retention under TIF in a similar study (Fennimore and Ajwa, 2011). We used a 9.8 eV (ionization energy) lamp which does not have a response to Pic, only DMDS was detected by the lamp. The maximum readable concentration for the MiniRAE 3000 is 3000 ppm DMDS. A

particle filter was attached to the end of the VOC meter probe to prevent soil particles and moisture from entering the meter. VOC readings were taken every other day after the fumigants started to dissipate until planting. Sampling earlier when fumigant concentrations are extremely high can damage internal sensors in the equipment and confound subsequent readings. Therefore, periodic samples were taken to determine when fumigant concentrations began decreasing and then sequential sampling was initiated. VOC sampling was performed during the afternoon on sampling dates. Samples were taken midway between the bed center and the bed shoulder. A 1.3 cm diameter round wooden dowel was inserted vertically to a depth of 10 cm. The dowel was removed and the probe was immediately inserted into the headspace. A seal was formed between the mulch and particle filter and readings continued until values stabilized or decreased and maximum values were recorded. Three sub-samples were taken from each plot and averaged. Sampling holes were covered with Poly Tarp Tape (BAC Industries Inc., Minneapolis, MN, USA) after measurement to prevent gas escape. Subsequent samples were taken from separate sampling holes.

Soil temperatures under the plastic were measured using two S-TMB-M002 12-bit Temperature/RH Smart Sensors (Onset Computer Corp., Bourne, MA, USA). These sensors were attached to a HOBO micro station data logger (Onset Computer Corp., Bourne, MA, USA), which recorded and stored the data. Temperatures were measured within the fumigated zone at depths of 5 cm and 20 cm. The data logger took measurements were taken every 30 seconds and averaged them every two minutes. Temperatures at both depths are reported for 8 am and 3 pm. These represent the daily maximum and minimum temperatures.

Data Analysis

The retention data was transformed using a log transformation. Statistical differences between treatment means were determined by analysis of variance (ANOVA) by day after fumigation (DAF). Significant differences between treatment means were separated using Duncan's multiple range test at $P \leq 0.05$.

Results

Fall 2009

Soil temperatures ranged from 21 to 40°C (Fig 6.1). Average low soil temperature at 8 am was 25°C at 5 cm and 27°C at 20 cm. Average high soil temperature at 3 pm was 35°C at 5 cm and 30°C at 20 cm. Based upon the low soil temp at 20 cm (27°C) the labeled plant back period was 21 DAF (Table 6.1). The 468 L/ha rate drip applied under TIF had a higher concentration at plant back (17.9 ppm) than the same rate shank applied under VIF (1.7 ppm), however it probably would not be detrimental to a crop. The drip applied 468 L/ha treatment under TIF was retained at a greater concentration than the drip applied 561 L/ha treatment and the 468 L/ha shank treatment under VIF (Fig 6.2). The treatments under VIF (468 L/ha shank applied and 561 L/ha drip applied) had similar retention concentrations, except at 25 DAF.

Spring 2010

Temperatures within the soil ranged from 11 to 43°C (Fig 6.3). Mean low soil temperature at 8 am was 18°C at 5 cm and 20°C at 20 cm. Average high soil temperature at 3 pm was at 5 cm was 35°C and at 20 cm was 25°C. The shank applied treatment was applied a week before the drip applied treatments and VOC measurements were recorded on the same dates for all treatments. Therefore, VOC readings for the shank treatment were delayed by a week in terms of DAF (Fig 6.4). The 468 L/ha rate drip applied under TIF was retained at a greater concentration at 10 DAF. At 11 DAF the 374 L/ha drip applied TIF treatment

concentration was similar compared to the 468 L/h drip applied TIF treatment and the 561 L/ha drip applied VIF treatment. The shank applied 468 L/ha VIF treatment fumigant concentrations were maintained at the greatest level compared to the other treatments from 17 to 21 DAF. Meanwhile, the drip applied treatment under VIF (561 L/ha) was retained at the least concentration throughout the experiment, although not always significantly lesser than the drip applied 374 L/ha and 468 L/ha under TIF treatments. The plant back period for spring 2010 was 28 DAF based on average low soil temperatures at 20 cm (Table 6.1). At 28 DAF all the drip applied treatments were 0 ppm. The shank applied treatment had DMDS concentrations of 15.8 ppm at plant back.

Fall 2010

Soil temperatures ranged between 19 and 41°C (Fig 6.5). Average low soil temperature at 8 am was 25°C at 5 cm and 27°C at 20 cm. Mean high soil temperature at 3 pm was 38°C at 5 cm and 33°C at 20 cm. The plant back period for fall 2010 was 21 DAF based on average low soil temperatures at 20 cm (Table 6.1). At 21 DAF all the drip applied treatments were 0 ppm and the shank treatment was 4.6 ppm. The drip applied 561 L/ha rate under VIF was retained at the least concentration until 11 DAF (Fig 6.6). The shank applied 468 L/ha rate under VIF was retained at similar concentrations as the drip applied 374 L/ha and 468 L/ha rates under TIF until 13 DAF. Fumigant retention concentrations were consistently greater for the shank applied 468 L/ha under VIF treatment compared to the drip applied 561 L/ha under VIF treatment throughout the measurement period.

Spring 2011

Temperatures ranged between 13 and 43°C within the soil (Fig 6.7). Mean low temperature at 8 am was 20°C at 5 cm and 22°C at 20 cm. Average high temperature at 3 pm was 34°C at 5 cm and 25°C at 20 cm. The plant back period for spring 2011 was 21 DAF based on average low soil temperatures at 20 cm (Table 6.1). At 21 DAF all the drip applied treatments had DMDS concentrations ≤ 3.1 ppm and the shank applied treatment had concentrations of 36.7 ppm. The temperatures were barely above the threshold for a shorter plant-back period which might explain the high DMDS concentration in the shank treatment. Between 17 and 24 DAF fumigant concentration in the shank applied 468 L/ha VIF treatment was greater than all other treatments (Fig 6.8). At 10 DAF, both the drip applied TIF treatments (374 and 468 L/ha) were retained at greater concentrations than the drip applied 561 L/ha VIF treatment.

Fall 2011

Soil temperatures ranged from 20 to 39°C (Fig 6.9). Average low soil temperature at 8 am was 25°C at 5 cm and 27°C at 20 cm. Mean high soil temperature at 3 pm was 34°C at 5 cm and 29°C at 20 cm. The plant back period for fall 2011 was 21 DAF based on average low soil temperatures at 20 cm (Table 6.1). At 21 DAF all the drip applied treatments were 0 ppm DMDS and the concentration for the shank treatment was 5 ppm DMDS. The fumigant concentration for drip applied treatments under TIF (374 and 468 L/ha) were similar throughout the experiment (Fig 6.10). The drip applied DMDS under TIF (374 and 468 L/ha) was retained at a greater concentration than the drip applied DMDS under VIF (561 L/ha). The shank applied DMDS under VIF (468 L/ha) was retained at similar concentrations as the drip applied 561 L/ha under VIF until 9 DAF. After 9 DAF the shank applied DMDS was retained at equal or greater concentrations than the other treatments.

Discussion

Drip applied DMDS retention can be increased with TIF compared to VIF. Increased fumigant retention resulted in the ability to reduce application rates while maintaining similar fumigant concentration. Numerous studies have shown that VIF and TIF are significantly less permeable to fumigants than LDPE and HDPE (Gamliel et al., 1998a; Gamliel et al., 1998b; Gamliel et al., 1997; Wang et al., 1998; Yates et al., 2002; Ou et al., 2007; Santos et al., 2007; Wang et al., 1997; Chellemi et al., 2011; Gao et al., 2011a; Gao et al., 2011b; Qin et al., 2011; Fennimore and Ajwa, 2011). Based on our results, labeled plant back periods do not need to be extended for drip applied DMDS under TIF. Generally, applying DMDS through the drip irrigation resulted in lesser fumigant concentrations for a shorter period of time compared to the shank applied DMDS. Perhaps the small perforation in the mulch from emerged nutsedge in the drip applied treatments account for the lesser retention concentrations. Kim et al. (2003) found emissions in non-tarped soil columns were more rapid and produced greater maximum instantaneous flux in a shallow subsurface (10 cm) drip application of Telone EC compared to a deeper (30 cm) shank injection of Telone II. DMDS was retained for a longer period in the spring when temperatures were cooler and for a shorter period in the fall when temperatures were warmer. Various studies have found that film permeability, fumigant emissions and fumigant degradation increase with increasing temperatures (Papiernik and Yates, 2002; Wang et al., 1998; Gan et al., 1999; Ma et al., 2001; Guo and Gao, 2009). During spring the average low soil temperature at 20 cm (21.73°C) was barely above the threshold level (21.7°C), which resulted in a shorter plant back period (21 DAF compared to 28 DAF). The shorter plant back period could explain why the concentration of DMDS (36.7 ppm) was so great under the shank applied VIF treatment.

Conclusions

The TIF retained drip applied fumigants at greater concentrations than VIF. Drip applied fumigant rates can be reduced by approximately 33% from 561 L/ha under VIF to 374 L/ha under TIF while maintaining similar DMDS fumigant concentrations. Fumigant concentrations were generally lower in drip applied DMDS treatments compared to shank applied DMDS under VIF, except during fall 2011. In addition, plant-back period may be reduced for drip applied DMDS compared to shank applied applications. Higher soil temperatures in fall experiments reduced plant-back periods, while cooler temperatures in the spring led to longer plant-back periods. However, plant-back periods never exceeded 28 DAF for either season. Shank applied DMDS under VIF seems to be especially dependent upon temperature. During the spring seasons, the shank applied treatment under VIF was clearly retained at the greatest concentration for the longest period of time, however in the fall seasons it appears the shank treatment under VIF was retained at similar or lesser concentrations as the same rate (468 L/ha) drip applied under TIF. Reduced drip applied application rates under TIF result in decreased buffer zone distances, for example in a 16 ha field for a 374 L/ha rate the buffer zone would be reduced to 21 m from 46 m for a 561 L/ha rate.

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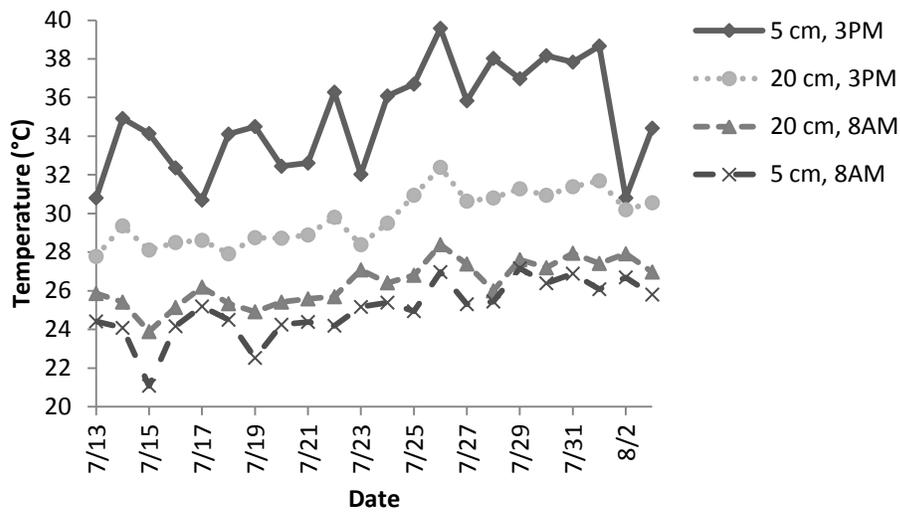


Fig. 6.1. Soil temperatures at 5 cm and 20 cm depths at 8 am and 3 pm under virtually impermeable film (VIF) mulch in a drip applied dimethyl disulfide (DMDS) experiment. Experiments were performed during the fall of 2009 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA.

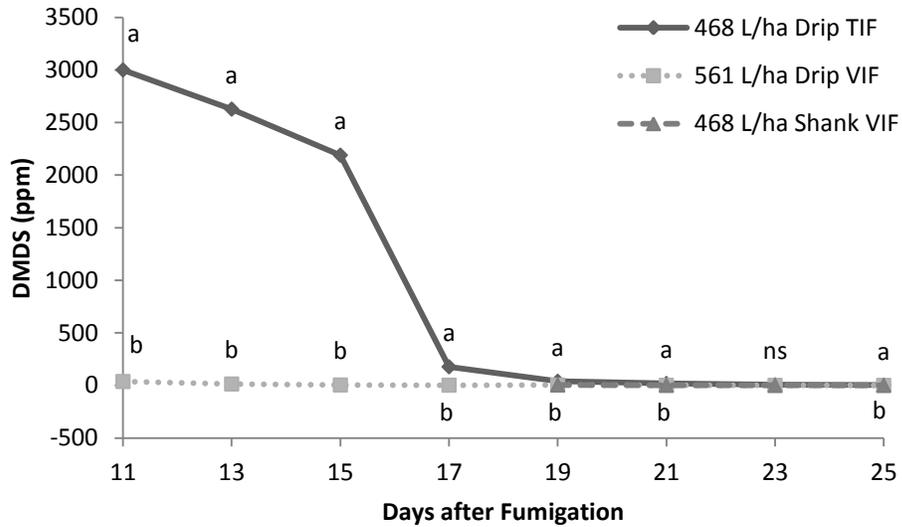


Fig. 6.2. Retention of drip applied dimethyl disulfide (DMDS) under virtually impermeable film (VIF) and totally impermeable film (TIF) mulches. Experiments were performed during fall 2009 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA. Means followed by the same letter are not significantly different at $P \leq 0.05$ by Duncan's multiple range test. Means are compared within the same day.

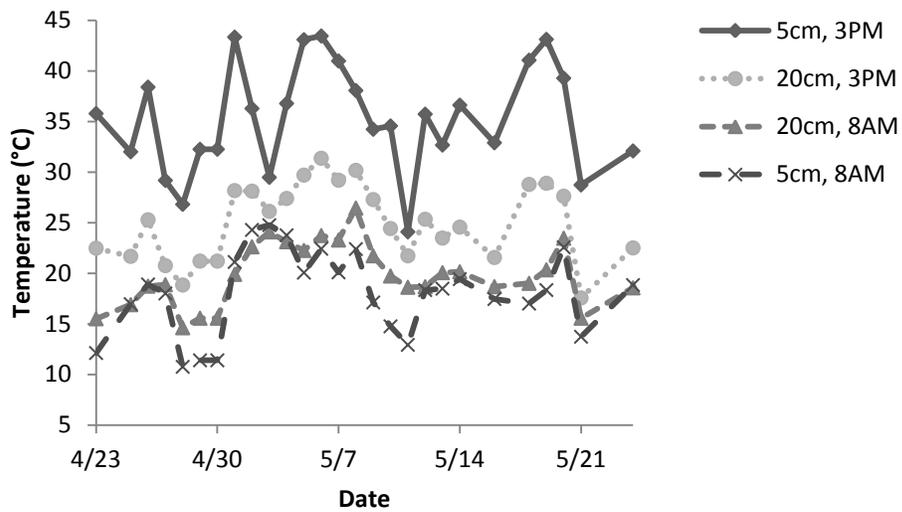


Fig. 6.3. Soil temperatures at 5 cm and 20 cm depths at 8 am and 3 pm under virtually impermeable film (VIF) mulch in a drip applied dimethyl disulfide (DMDS) experiment. Experiments were performed during the spring of 2010 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA.

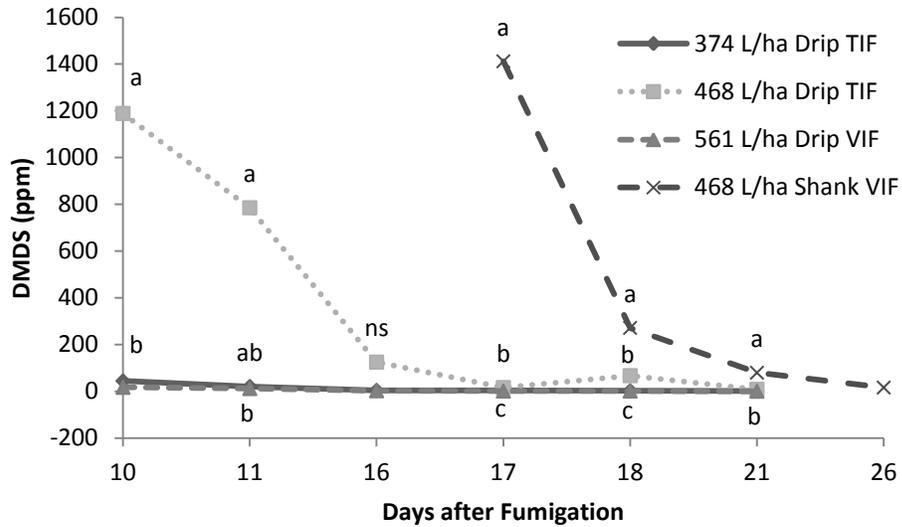


Fig. 6.4. Retention of drip applied dimethyl disulfide (DMDS) under virtually impermeable film (VIF) and totally impermeable film (TIF) mulches. Experiments were performed during spring 2010 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA. Means followed by the same letter are not significantly different at $P \leq 0.05$ by Duncan's multiple range test. Means are compared within the same day.

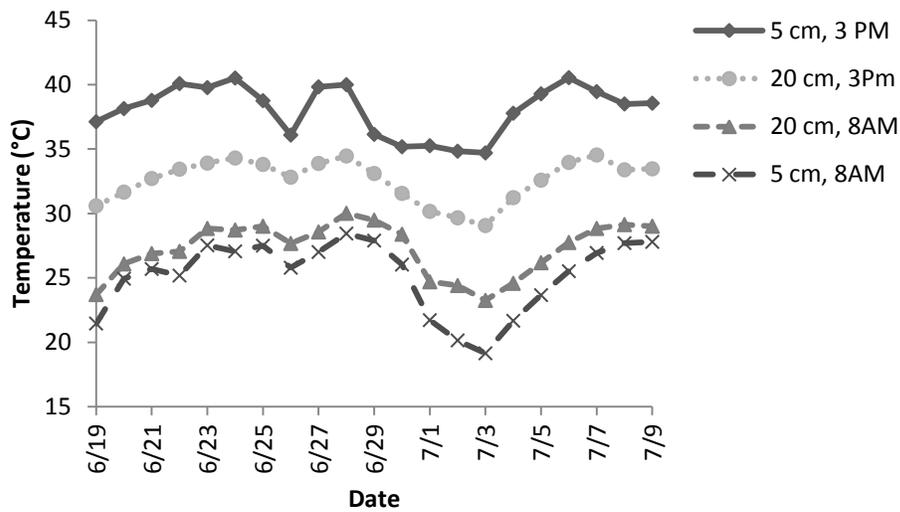


Fig. 6.5. Soil temperatures at 5 cm and 20 cm depths at 8 am and 3 pm under virtually impermeable film (VIF) mulch in a drip applied dimethyl disulfide (DMDS) experiment. Experiments were performed during fall 2010 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA.

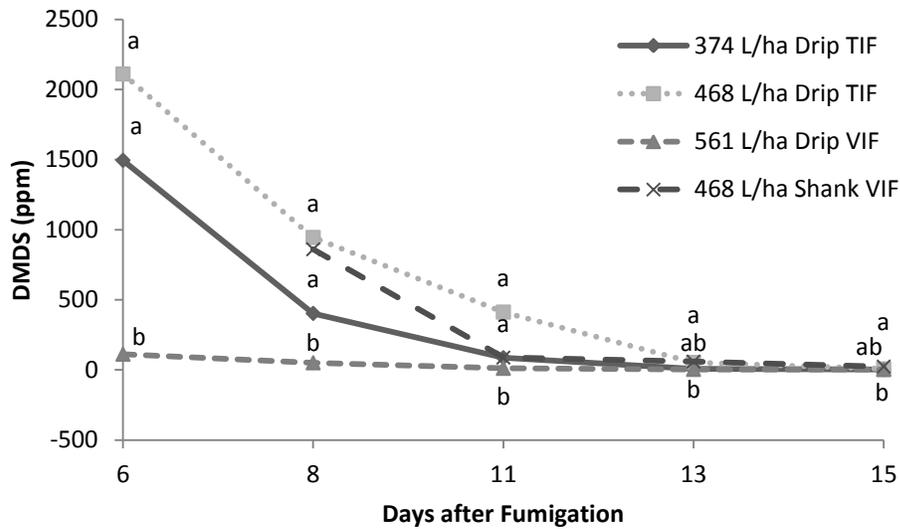


Fig. 6.6. Retention of drip applied dimethyl disulfide (DMDS) under virtually impermeable film (VIF) and totally impermeable film (TIF) mulches. Experiments were performed during fall 2010 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA. Means followed by the same letter are not significantly different at $P \leq 0.05$ by Duncan's multiple range test. Means are compared within the same day.

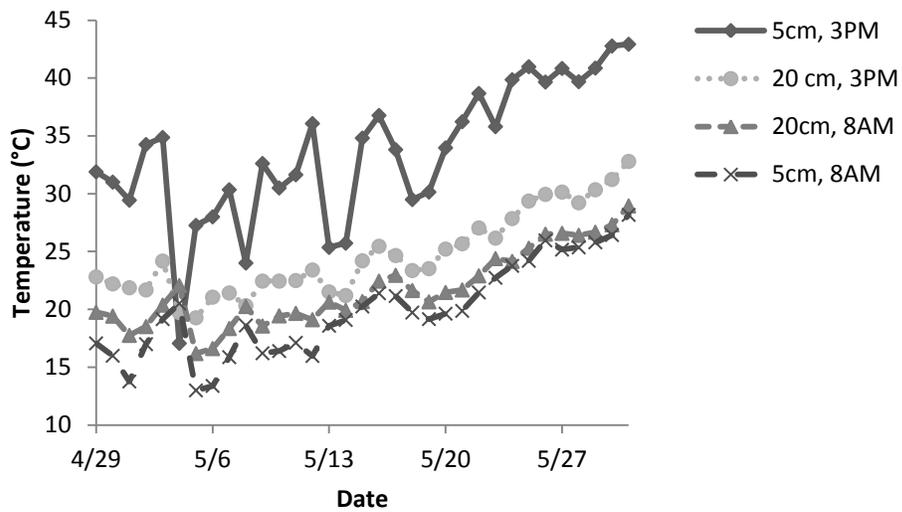


Fig. 6.7. Soil temperatures at 5 cm and 20 cm depths at 8 am and 3 pm under virtually impermeable film (VIF) mulch in a drip applied dimethyl disulfide (DMDS) experiment. Experiments were performed during spring 2011 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA.

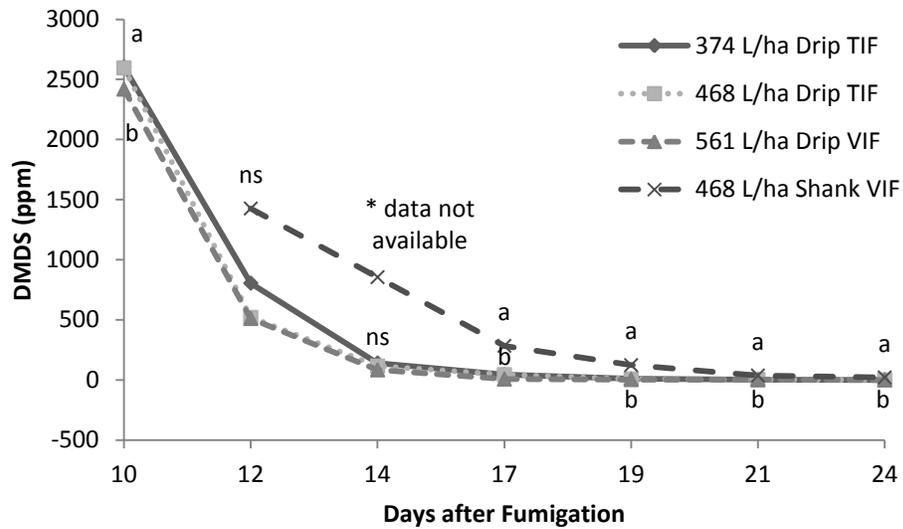


Fig. 6.8. Retention of drip applied dimethyl disulfide (DMDS) under virtually impermeable film (VIF) and totally impermeable film (TIF) mulches. Experiments were performed during spring 2011 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA. Means followed by the same letter are not significantly different at $P \leq 0.05$ by Duncan's multiple range test. Means are compared within the same day.

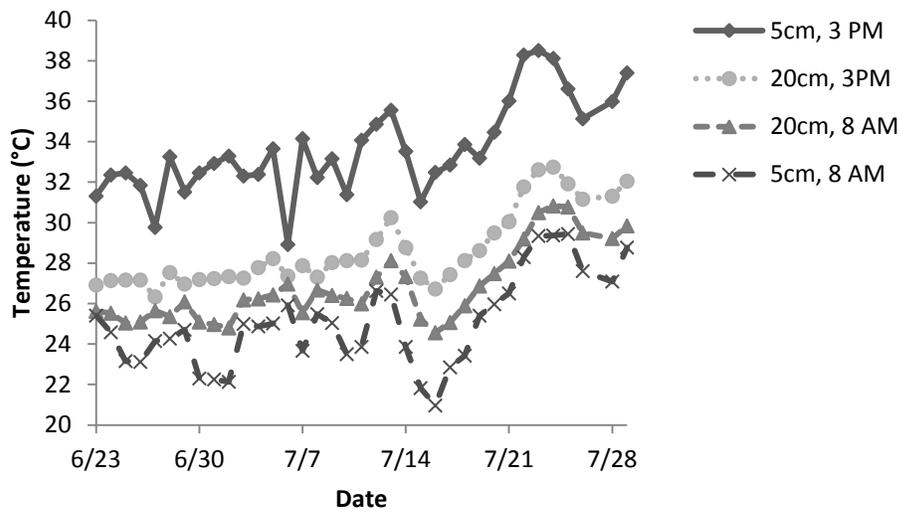


Fig. 6.9. Soil temperatures at 5 cm and 20 cm depths at 8 am and 3 pm under virtually impermeable film (VIF) mulch in a drip applied dimethyl disulfide (DMDS) experiment. Experiments were performed during fall 2011 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA.

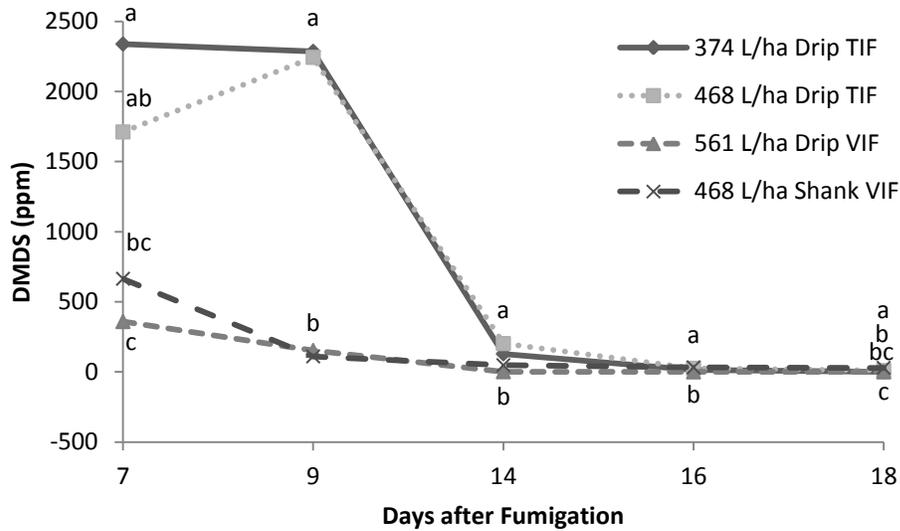


Fig. 6.10. Retention of drip applied dimethyl disulfide (DMDS) under virtually impermeable film (VIF) and totally impermeable film (TIF) mulches. Experiments were performed during fall 2011 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA. Means followed by the same letter are not significantly different at $P \leq 0.05$ by Duncan's multiple range test. Means are compared within the same day.

Table 6.1 Dimethyl disulfide (DMDS) concentrations (ppm) under virtually impermeable film (VIF) and totally impermeable film (TIF) mulch at labeled planting intervals in shank applied DMDS experiments. Labeled planting intervals are expressed as days after fumigation (DAF). Plant-back intervals are based upon average low soil temperatures at 20 cm when used with approved labeled VIF and metalized mulches. DMDS is not currently labeled for use with Vaporsafe TIF.

Season	Treatment	Labeled Plant-Back Interval (DAF)	DMDS Concentration(ppm) at Plant-Back
Fall 2009	468 L/ha Drip TIF	21	18
	561 L/ha Drip VIF	21	0.7
	468 L/ha Shank VIF	21	1.7
Spring 2010	374 L/ha Drip TIF	28	0
	468 L/ha Drip TIF	28	0
	561 L/ha Drip VIF	28	0
	468 L/ha Shank VIF	28	16
Fall 2010	374 L/ha Drip TIF	21	0
	468 L/ha Drip TIF	21	0
	561 L/ha Drip VIF	21	0
	468 L/ha Shank VIF	21	4.6
Spring 2011	374 L/ha Drip TIF	21	1.7
	468 L/ha Drip TIF	21	3.1
	561 L/ha Drip VIF	21	1.0
	468 L/ha Shank VIF	21	37
Fall 2011	374 L/ha Drip TIF	21	0
	468 L/ha Drip TIF	21	0
	561 L/ha Drip VIF	21	0
	468 L/ha Shank VIF	21	5.0

Chapter 7 Effect of Totally Impermeable Film on Dimethyl Disulfide Efficacy for Yellow Nutsedge Control in Fresh Market Tomato

Introduction

The primary weed controlled by methyl bromide (MBr) in tomato production on the Eastern Shore of Virginia is yellow nutsedge (*Cyperus esculentus* L.). In the southern United States, yellow nutsedge is among the most common and troublesome weeds in fruiting vegetables in Alabama, Georgia, Kentucky North Carolina and South Carolina (Webster, 2006). Although purple nutsedge (*Cyperus rotundus* L.) is a devastating weed of tomatoes in many areas it does not tolerate the temperate climate of Virginia. Yellow nutsedge is not completely controlled by plastic mulch, because the plant possesses sharp leaf tips that readily puncture and emerge through the plastic. Black mulch does suppress yellow nutsedge spread in terms of shoot production and lateral expansion compared to a non-mulched control (Webster, 2005). It is estimated that a single yellow nutsedge tuber produced 62 shoots by 24 weeks after planting compared to 208 shoots produced in the non-mulched control during the same time period.

Relatively low infestations of yellow nutsedge can result in decreased tomato yields. Stall and Morales-Payan (2003) found that season long interference of 25 yellow nutsedge plants/m² resulted in a 10% marketable yield loss of tomato. In addition, the critical weed free period for yellow nutsedge in tomato is between 2 – 10 weeks after transplanting to avoid tomato yield losses above 5%. The objective of this study was to test the effect of TIF and VIF on the efficacy of reduced rates of dimethyl disulfide (DMDS) to control yellow nutsedge in Virginia.

Materials and Methods

DMDS efficacy experiments were conducted at the Virginia Tech Eastern Shore Agricultural Research and Extension Center (ESAREC) in Painter, VA during the spring and fall of 2010, and 2011. Soil type at ESAREC is a Bojac sandy loam (Thermic Typic Hapludults) with 59% sand, 30% silt, and 11% clay with pH ranging from 6.2 to 6.5 and organic matter content of 0.50 to 0.75%. Soil was cultivated to a depth of 30 cm prior to fumigation. If necessary, overhead sprinkler irrigation was used to bring soil moisture to between 50 and 75% field capacity. A 79:21 w/w formulation of DMDS:chloropicrin (Pic) (United Phosphorus Inc., King of Prussia, PA, USA) fumigant was shank applied using a single row combination bed press 76 cm wide and 20 cm high with three back swept shanks. Shanks were 20 cm long and fumigant was released at the bottom of the shank. Experimental plots were 24 m long with a between row spacing of 1.8 m. Fumigant was applied on 8 April, 2010, 14 June, 2010, 2 May, 2011, and 14 June, 2011. Soil temperature at 20 cm prior to fumigant application was 13°C in the spring of 2010, 23°C in the fall of 2010, 21°C in the spring of 2011, and 24°C in the fall of 2011.

Efficacy of DMDS was evaluated with two mulches at various application rates. During all experiments, black (spring) or white on black (fall) Blockade® VIF (Berry Plastics Corp., Evansville, IN, USA) embossed polyethylene mulch with thickness 0.03 mm containing a nylon barrier was used. The TIF mulch used was black (spring), or white on black (fall) Vaporsafe® (Raven Industries Inc., Sioux Falls, SD, USA) polyethylene mulch with 0.05 mm thickness containing an EVOH barrier. The labeled rate for Paladin under VIF film in tomatoes to control nutsedge is 561 L/ha. The spring 2010 experiment treatments included an non-treated (non-fumigated) TIF, standard rate of DMDS:Pic (468 L/ha (535 kg/ha) broadcast) under VIF and TIF, a high rate (561 L/ha (642 kg/ha) broadcast) under VIF, and reduced rates (187 L/ha (214

kg/ha broadcast), 281 L/ha (321 kg/ha broadcast) and 374 L/ha (428 kg/ha broadcast)) under TIF. The experiment was repeated in the fall of 2010 and the spring and fall of 2011 with the addition of a non-treated (non-fumigated) VIF. Fumigant rates were adjusted with a Siemens® (Siemens Corporation, New York, NY, USA) flow meter (3.5 L/min of DMDS:Pic 79:21 at 100% flow), TeeJet® (TeeJet Technologies, Wheaton, IL, USA) flow regulators (orifice plates) and by varying tractor speed, as described by Gilreath et al. (2005). Experimental plots were arranged as a randomized complete block design with four replications.

Once the fumigant had dissipated, a single row of the tomato cultivar 'BHN 602' (BHNSeed, Immokalee, FL, USA) was transplanted into each plot. Experimental plots contained 25 plants spaced 46 cm apart within the row. Seedlings were transplanted on 21 May 2010, 13 July 2010, 13 June 2011, and 25 July 2011. Drip irrigation was provided to meet the water requirements of the crop. The crop was fertilized based on Cooperative Extension production recommendations (Wilson et al., 2012). Current recommended cultural and disease management practices for tomato in Virginia were implemented (Wilson et al., 2012).

Data Collection

Weed counts and disease prevalence (if present) were recorded at 1st Harvest. Tomatoes were harvested at the mature green stage on 30 July and 10 August for spring 2010, 29 September and 7 October for fall 2010, 23 August and 1 September for spring 2011, and 17 October and 26 October for fall 2011. Harvested tomato fruit were graded and sized according to the USDA standards for fresh market tomatoes (USDA, 1991). Yields combined from both harvests for each season are presented.

Data Analysis

Statistical differences in pathogen amounts and tomato yields between treatments were determined by analysis of variance (ANOVA). Significant differences between treatment means were separated using Duncan's multiple range test at $P \leq 0.05$.

Results

Spring 2010

All rates of DMDS under TIF (0 - 2.5 shoots/m²) and 468 L/ha under VIF (2.2 shoots/m²) controlled yellow nutsedge better than the non-treated TIF (5.4 shoots/m²) (Table 7.1). Applications of DMDS at rates of 281 L/ha (0.3 shoots/m²), 374 L/ha (0.2 shoots/m²), and 468 L/ha (0 shoots/m²) under TIF provided better nutsedge control than 561 L/ha under VIF (4.3 shoots/m²). The number of broadleaf weeds (common lambsquarter (*Chenopodium album* L.) and carpetweed (*Mollugo verticillata* L.)) was greatest in the non-treated TIF (0.5 plants/m²), followed by the 187 L/ha rate under TIF (0.3 plants/m²). The remaining DMDS treatments controlled broadleaf weeds similarly (0 - 0.1 plants/m²). DMDS fumigation at all rates under both plastic films managed grasses (large crabgrass (*Digitaria sanguinalis* L.) and goosegrass (*Eleusine indica* (L.) Gaertn.) (0 - 0.03 plants/m²) and diseases (*Fusarium oxysporum* f.sp. *radicis-lycopersici* Jarvis and Shoemaker) and southern blight (*Sclerotium rolfsii* Sacc.)). (4 - 15% disease incidence) better than the non-treated TIF. There were tomato yield differences in all fruit grades (Table 7.2). DMDS fumigation at all rates under both films resulted in higher marketable tomato yields (26196 – 40674 kg/ha) than the non-treated TIF (8667 kg/ha). Reduced rates of 187 L/ha (40674 kg/ha) and 281 L/ha (35981 kg/ha) under TIF provided higher marketable tomato yields than the VIF treatments (26196 – 27368 kg/ha).

Fall 2010

DMDS fumigation at all rates under both films controlled yellow nutsedge (0 -4 shoots/m²) better than the non-treated TIF (96 shoots/m²) (Table 7.3). In addition, the non-treated TIF (96 shoots/m²) managed nutsedge better than the non-treated VIF (256 shoot/m²). There was no yield difference for medium fruit. There were yield differences for large, extra-large and marketable fruits. All the fumigated treatments (38894 – 51891 kg/ha) and the non-treated TIF (36401 kg/ha) provided greater marketable tomato yields than the non-treated VIF (22103 kg/ha). The standard rate of 468 L/ha DMDS under VIF (54797 kg/ha) provided greater marketable tomato yields than 281 L/ha under TIF (38894 kg/ha) and the non-treated TIF (36401 kg/ha).

Spring 2011

DMDS applied at all rates under both films (0.03 -3.1 shoots/m²) and the non-treated TIF (6.6 shoots/m²) controlled yellow nutsedge better than the non-treated VIF (69 shoots/m²) (Table 7.4). There was no difference in nutsedge control between the non-treated TIF (6.6 shoots/m²) and DMDS fumigation (0.03 – 3.1 shoots/m²). There were no yield differences in medium and extra large fruit between treatments. There were differences in large and marketable tomato yields between treatments. DMDS fumigation at all rates under TIF (45176 -51294 kg/ha) and 561 L/ha under VIF (49641 kg/ha) resulted in greater marketable tomato yields than the non-treated VIF (33196 kg/ha). DMDS applied at 281 L/ha (49594 kg/ha) and 468 L/ha under TIF (51294 kg/ha) and at 561 L/ha under VIF (49641 kg/ha) provided higher marketable yields than the non-treated TIF (37553 kg/ha). All DMDS fumigant treatments had similar marketable tomato yields (43571 – 51294 kg/ha).

Fall 2011

All rates of DMDS under both films (0 – 5.8 shoots/m²) and the non-treated TIF (65 shoots/m²) provided a higher level of nutsedge control than the non-treated VIF (230 shoots/m²) (Table 7.5). There were yield differences between treatments for all tomato fruit sizes. DMDS fumigation at all rates under TIF (58545 – 61540 kg/ha) and VIF (54330 -57718 kg/ha) and the non-treated TIF (46829 kg/ha) resulted in greater marketable fruit yields than the non-treated VIF (20389 kg/ha). All the DMDS rates under TIF (58545 -61540 kg/ha) and 468 L/ha under VIF (57718 kg/ha) increased marketable yields compared to the non-treated TIF (46829 kg/ha). The non-treated TIF (46829 kg/ha) and 561 L/ha under VIF (54330 kg/ha) yielded similar amounts of marketable tomato fruit.

Discussion

This results indicate reduced rates of DMDS under TIF can be used to manage yellow nutsedge, broadleaves (common lambsquarter and carpetweed), grasses (large crabgrass and goosegrass) and diseases (fusarium wilt, fusarium crown rot and southern blight). DMDS has been shown to control yellow nutsedge under LDPE, metalized mulch and VIF (Olson and Rich, 2007). DMDS has also been shown to control purple nutsedge (*Cyperus rotundus* L.) and large crabgrass (Culpepper et al., 2008) and *Amaranthus* spp. and *Pythium* spp. (Othman et al., 2010). Resulting pest management from reduced rates of DMDS under TIF led to increased yields compared to the non-treated VIF and non-treated TIF in some cases. Rates of 1,3-D plus Pic could be reduced by 33% under TIF compared to HDPE, while maintaining similar weed control (yellow nutsedge, common purslane, and common chickweed) and strawberry fruit yield as a standard rate (392 kg/ha) of MBr under HDPE (Fennimore and Ajwa, 2011). DMDS fumigation has been used effectively to increase yields compared to an non-treated control or maintain yields compared to MBr controls in tomato (Olson and Rich, 2007; Welker et al, 2006),

cantaloupe (Olson and Rich, 2007), and strawberry (Othman et al., 2010; Welker et al., 2007; Lopez-Aranda et al., 2009; Garcia-Mendez et al., 2008).

Non-treated TIF resulted in lower yellow nutsedge densities than non-treated VIF which impacted tomato fruit yield. Webster et al. (2005) demonstrated that yellow nutsedge shoot production was reduced by black LDPE compared to a non-mulched control. Greater nutsedge densities were observed in the fall crop compared to the spring crop and this seems to have had a more detrimental impact on tomato yields in the absence of disease. This may contradict the findings of Taylerson (1967) and Tumbleson and Kommedahl (1962) that found yellow nutsedge germination to be highest in winter and spring and most dormant in late summer and fall. However, soilborne diseases and broadleaf weeds were primarily problematic in the spring crop.

Conclusions

Non-treated TIF results in lower nutsedge densities and higher tomato yields than non-treated VIF. It appears the EVOH barrier layer in TIF physically prevents nutsedge emergence to a greater degree than VIF mulch. Fumigation with DMDS under VIF and TIF can provide better nutsedge control and provide higher tomato yields than a non-treated VIF and non-treated TIF. DMDS rates under TIF can be reduced to 187 L/ha under TIF while still resulting in yellow nutsedge control and marketable yields similar or better than 468 L/ha and 561 L/ha under VIF. Thus TIF can reduce DMDS application rates by 60 to 67% while still providing effective control of yellow nutsedge. TIF may allow growers to reduce DMDS fumigant rates resulting in higher economic returns and decreased buffer zones, while maintaining yellow nutsedge control and increasing tomato yields.

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Table 7.1. Weed (yellow nutsedge, broadleaves, and grasses) and disease management in a shank applied dimethyl disulfide (DMDS) experiment. The primary species of broadleaves were common lambsquarter (*Chenopodium album* L.) and carpetweed (*Mollugo verticillata* L.), grasses were large crabgrass (*Digitaria sanguinalis* L.) and goosegrass (*Eleusine indica* L.) Gaertn.), and diseases were fusarium wilt (*Fusarium oxysporum* f.sp. *lycopersici* (Sacc.) W.C. Snyder and H.N. Hans), fusarium crown rot (*Fusarium oxysporum* f.sp. *radicis-lycopersici* Jarvis and Shoemaker) and southern blight (*Sclerotium rolfsii* Sacc.). Experiments were performed during spring 2010 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA.

Treatment	Weeds/m ²			Disease Incidence
	Nutsedge	Broadleaves	Grasses	% Mortality
Non-treated TIF	5.4 a ^z	0.5 a	0.3 a	42 a
187 L/ha TIF	2.5 bc	0.3 b	0.03 b	4 b
281 L/ha TIF	0.3 c	0.1 bc	0 b	15 b
374 L/ha TIF	0.2 c	0.03 c	0 b	12 b
468 L/ha TIF	0 c	0.03 c	0 b	5 b
468 L/ha VIF	2.2 bc	0 c	0 b	10 b
561 L/ha VIF	4.3 ab	0.09 c	0 b	12 b

^z Means followed by the same letter within a column are not significantly different at $P \leq 0.05$ based on Duncan's multiple range test.

Table 7.2. Tomato yield in a shank applied dimethyl disulfide (DMDS) experiment. Experiments were performed during spring 2010 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA.

Treatment	Yields (kg/ha)			
	Medium	Large	Extra-Large	Marketable
Non-treated TIF	2317 c ^z	3157 c	3192 e	8667 d
187 L/ha TIF	4752 abc	16687 a	19218 a	40674 a
281 L/ha TIF	5780 ab	13194 ab	17008 ab	35981 ab
374 L/ha TIF	6620 a	12827 ab	11099 cd	30546 bc
468 L/ha TIF	6167 ab	14047 ab	14101 bc	34313 abc
468 L/ha VIF	3599 bc	10604 b	13166 bcd	27368 c
561 L/ha VIF	6092 ab	11533 b	8571 d	26196 c

^z Means followed by the same letter within a column are not significantly different at $P \leq 0.05$ based on Duncan's multiple range test.

Table 7.3. Yellow nutsedge management and tomato yield in a shank applied dimethyl disulfide (DMDS) experiment. Experiments were performed during fall 2010 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA.

Treatment	Nutsedge/m ²	Yields (kg/ha)			
		Medium	Large	Extra-Large	Marketable
Non-treated VIF	256 a ^z	6485 ns	6180 c	9439 c	22103 c
Non-treated TIF	96 b	6410	9439 bc	20552 bc	36401 b
187 L/ha TIF	4.0 c	7610	12108 ab	29306 ab	49025 ab
281 L/ha TIF	1.4 c	7101	9649 abc	22144 ab	38894 b
374 L/ha TIF	0 c	8904	11499 ab	27328 ab	47730 ab
468 L/ha TIF	0 c	9446	13234 a	29212 ab	51891 ab
468 L/ha VIF	3.0 c	7914	12854 ab	34029 a	54797 a
561 L/ha VIF	0.2 c	7521	10855 ab	32505 ab	50880 ab

^z Means followed by the same letter within a column are not significantly different at $P \leq 0.05$ based on Duncan's multiple range test. ns=not significant.

Table 7.4. Yellow nutsedge management and tomato yield in a shank applied dimethyl disulfide (DMDS) experiment. Experiments were performed during spring 2011 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA.

Treatment	Nutsedge/m ²	Yields (kg/ha)			
		Medium	Large	Extra-Large	Marketable
Non-treated VIF	69 a ^z	5387 ns	12983 c	14826 ns	33196 c
Non-treated TIF	6.6 b	6864	17062 abc	13627	37553 bc
187 L/ha TIF	0.2 b	4892	16161 bc	24123	45176 ab
281 L/ha TIF	0.8 b	4560	19393 ab	25640	49594 a
374 L/ha TIF	0.03 b	4770	19406 ab	21697	45874 ab
468 L/ha TIF	0.08 b	7460	22645 a	21189	51294 a
468 L/ha VIF	3.1 b	5536	17651 abc	20382	43571 abc
561 L/ha VIF	1.0 b	7521	20436 ab	21683	49641 a

^z Means followed by the same letter within a column are not significantly different at $P \leq 0.05$ based on Duncan's multiple range test. ns= not significant.

Table 7.5. Yellow nutsedge management and tomato yield in a shank applied dimethyl disulfide (DMDS) experiment. Experiments were performed during fall 2011 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA.

Treatment	Nutsedge/m ²	Yields (kg/ha)			
		Medium	Large	Extra-Large	Marketable
Non-treated					
VIF	231 a ^z	2954 d	7128 c	10306 b	20389 c
Non-treated TIF	64 b	5583 c	16107 b	25139 a	46829 b
187 L/ha TIF	0 b	9798 a	21920 a	27951 a	59669 a
281 L/ha TIF	0.03 b	9405 a	21521 a	27619 a	58545 a
374 L/ha TIF	0.4 b	8436 ab	21331 a	31773 a	61540 a
468 L/ha TIF	0.03 b	7325 bc	21968 a	31271 a	60564 a
468 L/ha VIF	0.5 b	8660 ab	18526 ab	30533 a	57718 a
561 L/ha VIF	5.8 b	8077 ab	18885 ab	27368 a	54330 ab

^z Means followed by the same letter within a column are not significantly different at $P \leq 0.05$ based on Duncan's multiple range test.

Chapter 8 Effect of Totally Impermeable Film on Methyl Iodide Efficacy for Yellow Nutsedge Control in Fresh Market Tomato

Introduction

In the Southern United States, yellow nutsedge (*Cyperus esculentus* L.) is among the most common and troublesome weeds in fruiting vegetables in Alabama, Georgia, Kentucky North Carolina and South Carolina (Webster, 2006). Yellow nutsedge is not completely controlled by plastic mulch, because the plant possesses sharp leaf tips that readily puncture and emerge through the plastic. Black mulch does suppress yellow nutsedge spread in terms of shoot production and lateral expansion compared to a non-mulched control (Webster, 2005). It is estimated that a single yellow nutsedge tuber produced 62 shoots by 24 weeks after planting compared to 208 shoots produced in the non-mulched control during the same time period.

Relatively low infestations of yellow nutsedge can result in decreased tomato yields. Stall and Morales-Payan (2003) found that season long interference of 25 yellow nutsedge plants/m² resulted in a 10% marketable yield loss of tomato. In addition, the critical weed free period for yellow nutsedge in tomato is between 2 – 10 weeks after transplanting to avoid tomato yield losses above 5%. MeI is very costly compared to other soil fumigants; therefore reduced application rates would be beneficial to growers (Gilreath and Santos, 2011). The objective of this experiment was to test the efficacy of reduced rates of MeI in combination with TIF on yellow nutsedge and the effect on tomato yield.

Materials and Methods

MeI efficacy experiments were conducted at the Virginia Tech Eastern Shore Agricultural Research and Extension Center (ESAREC) in Painter, VA during the spring and fall of 2010, and 2011. Soil type at ESAREC is a Bojac sandy loam (Thermic Typic Hapludults) with 59% sand, 30% silt, and 11% clay with pH ranging from 6.2 to 6.5 and organic matter content of 0.50 to 0.75%. Soil was cultivated to a depth of 30 cm prior to fumigation. If necessary, overhead sprinkler irrigation was used to bring soil moisture capacity to between 50 and 75% field capacity. The fumigant formulation used was MeI:chloropicrin (Pic) 50:50 (w/w). The fumigant was shank applied using a single row combination bed press 76 cm wide and 20 cm high with three back swept shanks. Shanks were 20 cm long and fumigant was released at the bottom of the shank. Experimental plots were 24 m long with a between row spacing of 1.8 m.

The treatments in the experiment were a non-treated (non-fumigated) control utilizing TIF, a standard rate for highly retentive films (93.3 L/ha (178 kg/ha broadcast)) under VIF and TIF, and reduced rates (37.3, 56, 74.6 L/ha (71.2, 106.8, 142.5 kg/ha broadcast)) under TIF. Experimental plots were arranged as a randomized complete block design with four replications. Black films were used in the spring and white on black were used in the fall seasons. The mulch types used were Blockade® VIF (Berry Plastics Corp., Evansville, IN, USA) embossed polyethylene mulch with thickness 0.03mm containing a nylon barrier and Vaporsafe® TIF (Raven Industries Inc., Sioux Falls, SD, USA) polyethylene mulch with 0.05 mm thickness containing an EVOH barrier. Pliant Blockade is currently labeled for use with Midas 50:50, while Vaporsafe® TIF is not. The labeled fumigant rate for states other than Florida under highly retentive tarps in tomato to control nutsedge is a minimum of 94.4 L/ha (179.2 kg/ha broadcast). Fumigant application rates were adjusted by flow rate (measured by King® flow meter (King Instrument Company, Garden Grove, CA, USA) using a 10W float (0.75 L/min of

water at 100% flow)) and tractor speed. In order to achieve uniform fumigant delivery between chisels in the bed using low fumigant rates, a small diameter tubing (1.6mm) was used, and lines were fully charged before fumigating plots as described by Gilreath et al. (2005). Experiments were fumigated on 15 April 2010, 18 June 2010, 27 April 2011, and 11 August 2011.

These experiments were planted on 11 May 2010, 13 July 2010, 17 May 2011, and 22 August 2011. Once the fumigant had dissipated the beds were planted with a crop. Tomato cultivar 'BHN 602' (BHNSeed, Immokalee, FL, USA) was planted during every experiment, except in the fall of 2011. Adverse weather conditions prohibited timely fumigant application during the fall 2011. This delayed planting beyond the date necessary for tomato. In order to maintain as much similarity between seasons, a broccoli crop was established to maintain fertigation and irrigation effects on nutsedge growth. Experimental plots contained 25 plants spaced 46 cm apart within the row. Drip irrigation was provided to meet the water requirements of the crop. The crop was fertilized based on Cooperative Extension production recommendations (Wilson et al., 2012). Current recommended cultural and disease management practices for tomato in Virginia were implemented (Wilson et al., 2012).

Data Collection

Tomatoes were harvested twice per season at the mature green stage. Broccoli was harvested once. Harvested tomato fruit were graded and sized according to the USDA standards for grades of fresh tomatoes (USDA, 1991). Tomato yields combined from both harvests for each season are presented. In the spring of 2010 tomatoes were picked on 21 July and 30 July, in the fall of 2010 tomatoes were harvested on 29 September and 7 October, in the spring of 2011 tomatoes were harvested on 27 July and 5 August, and in the fall of 2011 broccoli was harvested on 17 October. Yellow nutsedge counts were taken at harvest. Yellow nutsedge counts were taken on 21 July 2010, 20 September 2010, 2 August 2011, and 24 October 2011.

Data Analysis

Statistical differences in disease incidence, nutsedge populations, and crop yields between treatments were determined by analysis of variance (ANOVA). Significant differences between treatment means were separated using Duncan's multiple range test at $P \leq 0.05$.

Results

Spring 2010

There were differences between weed control and disease management between treatments (Table 8.1). A standard MeI rate under both films (0 – 0.03 shoots/m²) and reduced rates under TIF (0 – 0.05 shoots/m²) controlled yellow nutsedge better than the non-treated TIF (1.9 shoots/m²). There were no difference in yellow nutsedge control (0 -0.05 shoots/m²) between MeI fumigated plots. In addition, all MeI fumigation provided a superior broadleaf, grass, and disease management compared to the non-treated TIF. There were no yield differences between treatments for medium, extra-large, and marketable sized fruits (Table 8.2). There were yield differences for large sized fruit. MeI applied at a standard rate (93.3 L/ha) under TIF resulted in higher large fruit yields (14758 kg/ha) than the lowest rate (37.3 L/ha) applied under TIF (11153 kg/ha). All fumigant treatments (12454 – 14758 kg/ha), except the 37.3 L/ha rate under TIF (11153 kg/ha), produced greater large fruit yield than the non-treated TIF (9317 kg/ha).

Fall 2010

There were differences in nutsedge management between treatments (Table 8.3). MeI applied at the labeled rate under both films and at reduced rates under TIF (0.2 – 8.8 shoots/m²)

provided better yellow nutsedge management than the non-treated TIF (130 shoots/m²). There were no yield differences in medium and large sized fruits between treatments. There were yield differences in extra-large and marketable sized fruits between treatments. MeI applied at a standard rate under both films and at all reduced rates under TIF provided greater extra-large (22863-29219 kg/ha), and marketable tomato yields (40507 – 50211 kg/ha) compared to the non-treated TIF (13498 and 26860, respectively).

Spring 2011

There were differences in yellow nutsedge and grass management between treatments (Table 8.4). There was no difference in broadleaf control between treatments. Reduced rates of methyl bromide under TIF and the labeled rate under both films controlled yellow nutsedge (0 - 0.1 shoots/m²) better than the non-treated TIF (3.8 shoots/m²). The lowest rate under TIF had similar grass populations as the non-treated TIF. All the higher MeI rates (56 – 93.3 L/ha) provided similar grass control as the non-treated TIF and better grass control than 37.3 L/ha under TIF. There were no differences in yield for any fruit size category between treatments (Table 8.5). It appears the low nutsedge densities encountered, even in the non-treated TIF, did not have a significant impact on tomato yield.

Fall 2011

All MeI treatments (3.3 – 22 shoots/m²) controlled yellow nutsedge better than the non-treated TIF (49 shoots/m²) (Table 8.6). The standard rate (93.3 L/ha) applied under TIF (3.3 shoots/m²) provided better nutsedge control than the lowest rate (37.3 L/ha) under TIF (49 shoots/m²). There were significant differences in yield (broccoli number and weight) between treatments. MeI applied under TIF yielded a greater number of broccoli heads (1579 -1763 heads/ha) than the non-treated TIF (1230 heads/ha). Reduced MeI rates (37.3 L/ha, 56 L/ha, and 74.6 L/ha) under TIF resulted in greater number of broccoli heads (1653 – 1763 heads/ha) than the labeled rate (93.3 L/ha) under VIF (1341 heads/ha). MeI applied under TIF at various rates resulted in higher early broccoli yields (4526 – 4983 kg/ha) than the labeled rate under VIF (93.3 L/ha) (3451 kg/ha). Fumigation with MeI at a standard rate under both films (3451 -4526 kg/ha) and at reduced rates under TIF (4853 – 4983 kg/ha) provided higher early broccoli yields than the non-treated TIF (2462 kg/ha).

Discussion

TIF can be used with reduced rates of MeI while providing acceptable yellow nutsedge control and tomato and broccoli yields. Several laboratory and field studies have shown MeI to be as or more effective than MBr at controlling yellow nutsedge (Hutchinson et al., 2003; Zang et al., 1997; Olson and Kreger, 2007; Gilreath and Santos, 2011). Furthermore, MeI is very costly compared to other soil fumigants, therefore reduced application rates and formulations with lesser MeI proportions would be beneficial to growers (Gilreath and Santos, 2011). Field experiments have shown reduced MeI rates under VIF mulch and formulations with reduced MeI amounts can result in tomato yields similar to MBr (Olson and Kreger, 2007; Gilreath and Santos 2011). Rates of 1,3-D plus Pic could be reduced by 33% under TIF compared to HDPE, while maintaining similar weed control (yellow nutsedge (*Cyperus esculentus* L.), common purslane (*Portulaca oleracea* L.), and common chickweed (*Stellaria media* L.)) and strawberry fruit yield as a standard rate (392 kg/ha) of MBr under HDPE (Fennimore and Ajwa, 2011). In the fall 2011 yellow nutsedge numbers were higher in the MeI treated plots than in previous seasons. Poor weed control during that season was likely a result of heavy rainfall compared to the other seasons. Also, during the fall 2011 it appears broccoli may be superior at suppressing yellow

nutsedge populations compared to tomato, when populations from both fall seasons are compared. This is likely due to shading from the growth habit of broccoli (horizontal leaf orientation) and the competitive advantage broccoli (cool season crop) had in the cooler late fall temperatures. Heavier nutsedge densities were observed in the fall crop compared to the spring crop and this seems to have had a more detrimental impact on tomato yields. This may contradict the findings of Taylerson (1967) and Tumbleton and Kommedahl (1962) that found yellow nutsedge germination to be highest in winter and spring and most dormant in late summer and fall.

Conclusions

MeI rates could be reduced from 93.3 L/ha under VIF to 37.3 L/ha under TIF (60% rate reduction) while providing similar nutsedge control and vegetable yields. MeI fumigation under both films increased yellow nutsedge control and tomato/broccoli yield compared to the non-treated TIF. TIF can reduce buffer zone requirements, application rates, and possibly lower costs when used with MeI while increasing nutsedge efficacy and vegetable yields. For example the buffer zone could be reduced from 42 m to 25 m if the application rate was lowered from 93.3 L/ha to 56 L/ha on an 8ha field.

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Table 8.1. Weed and disease management in a methyl iodide (MeI) experiment. Experiments were performed during spring 2010 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA.

Treatment	Weeds/m ²			Disease Incidence
	Nutsedge	Broadleaves	Grasses	% Mortality
Non-treated TIF	1.9 a ^z	0.05 a	1.4 a	36 a
37.3 L/ha TIF	0.05 b	0 b	0.3 b	6 b
56 L/ha TIF	0 b	0 b	0 b	3 b
74.6 L/ha TIF	0 b	0.003 b	0 b	2 b
93.3 L/ha TIF	0 b	0.003 b	0.3 b	5 b
93.3 L/ha VIF	0.03 b	0.003 b	0 b	3 b

^z Means followed by the same letter within a column are not significantly different at $P \leq 0.05$ based on Duncan's multiple range test.

Table 8.2. Tomato yield in a methyl iodide (MeI) experiment. Experiments were performed during spring 2010 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA.

Treatment	Yield (kg/ha)			
	Medium	Large	Extra-Large	Marketable
Non-treated TIF	5780 ns	9317 c	21277 ns	36373 ns
37.3 L/ha TIF	4316	11153 bc	22821	38292
56.0 L/ha TIF	4859	12454 ab	22558	39870
74.6 L/ha TIF	5876	13220 ab	23425	42520
93.3 L/ha TIF	6681	14758 a	24631	46070
93.3 L/ha VIF	5861	14020 ab	29287	49168

^z Means followed by the same letter within a column are not significantly different at $P \leq 0.05$ based on Duncan's multiple range test. ns=not significant.

Table 8.3. Yellow nutsedge management and tomato yield in a methyl iodide (MeI) experiment. Experiments were performed during fall 2010 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA.

Treatment	Nutsedge/m ²	Yield (kg/ha)			
		Medium	Large	Extra-Large	Marketable
Non-treated					
TIF	130 a ^z	6322 ns	7040 ns	13498 b	26860 b
37.3 L/ha TIF	4.2 b	8558	10435	25695 a	44688 a
56.0 L/ha TIF	1.0 b	7677	9866	23194 a	40738 a
74.6 L/ha TIF	1.2 b	7467	10177	22863 a	40507 a
93.3 L/ha TIF	0.2 b	8091	10795	23092 a	41978 a
93.3 L/ha VIF	8.8 b	8484	12508	29219 a	50211 a

^z Means followed by the same letter within a column are not significantly different at $P \leq 0.05$ based on Duncan's multiple range test. ns=not significant.

Table 8.4. Weed management in a methyl iodide (MeI) experiment. Experiments were performed during spring 2011 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA.

Treatment	Weeds/m ²		
	Nutsedge	Grass	Broadleaves
Non-treated TIF	3.8 a	0.16 ab	0.2 ns
37.3 L/ha TIF	0.03 b	0.21 a	0.05
56 L/ha TIF	0 b	0 b	0.03
74.6 L/ha TIF	0 b	0 b	0
93.3 L/ha TIF	0.1 b	0 b	0
93.3 L/ha VIF	0.05 b	0 b	0.03

^z Means followed by the same letter within a column are not significantly different at $P \leq 0.05$ based on Duncan's multiple range test. ns=not significant.

Table 8.5. Yellow nutsedge management and tomato yield in a methyl iodide (MeI) experiment. Experiments were performed during spring 2011 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA.

Treatment	Yield (kg/ha)			
	Medium	Large	Extra-Large	Marketable
Non-treated TIF	6180 ns	18986 ns	37627 ns	62793 ns
37.3 L/ha TIF	6756	17828	37437	62021
56.0 L/ha TIF	7325	23459	37641	68424
74.6 L/ha TIF	7657	23506	38657	69820
93.3 L/ha TIF	6624	23770	35330	65724
93.3 L/ha VIF	7379	21460	39531	68370

^z Means followed by the same letter within a column are not significantly different at $P \leq 0.05$ based on Duncan's multiple range test. ns=not significant.

Table 8.6. Yellow nutsedge management and early broccoli yield (number and weight) in a methyl iodide (MeI) experiment. Experiments were performed during fall 2011 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA.

Treatment	Nutsedge/m ²	Broccoli Yield	
		Number/ha	Yield (kg/ha)
Non-treated TIF	49 a ^z	1230 c	2462 c
37.3 L/ha TIF	22 b	1653 a	4853 a
56.0 L/ha TIF	16 bc	1763 a	4871 a
74.6 L/ha TIF	13 bc	1708 a	4983 a
93.3 L/ha TIF	3.3 c	1579 ab	4526 a
93.3 L/ha VIF	19 bc	1341 bc	3451 b

^z Means followed by the same letter within a column are not significantly different at $P \leq 0.05$ based on Duncan's multiple range test.

Chapter 9 Effect of Totally Impermeable Film on Drip Applied Dimethyl Disulfide Efficacy for Yellow Nutsedge Control in Fresh Market Tomato

Introduction

In the Southern United States, yellow nutsedge (*Cyperus esculentus* L.) is among the most common and troublesome weeds in fruiting vegetables in Alabama, Georgia, Kentucky, North Carolina and South Carolina (Webster, 2006). Yellow nutsedge is not completely controlled by plastic mulch, because the plant possesses sharp leaf tips that readily puncture and emerge through the plastic. Black mulch does suppress yellow nutsedge spread in terms of shoot production and lateral expansion compared to a non-mulched control (Webster, 2005). It is estimated that a single yellow nutsedge tuber produced 62 shoots by 24 weeks after planting compared to 208 shoots produced in the non-mulched control during the same time period.

Relatively low infestations of yellow nutsedge can result in decreased tomato yields. Stall and Morales-Payan (2003) found that season long interference of 25 yellow nutsedge plants/m² resulted in a 10% marketable yield loss of tomato. In addition, the critical weed free period for yellow nutsedge in tomato is between 2 – 10 weeks after transplanting to avoid tomato yield losses above 5%. Drip application of fumigants results in less fumigant handlers and thus lowers respiratory equipment costs. The objective of this experiment was to test the efficacy of TIF with reduced rates of drip applied dimethyl disulfide (DMDS) on yellow nutsedge and the effect on tomato yield.

Materials and Methods

Drip applied DMDS fumigant efficacy experiments were conducted at the Virginia Tech Eastern Shore Agricultural Research and Extension Center (ESAREC) in Painter, VA during the fall of 2009, spring and fall of 2010, and spring and fall of 2011. Soil type at ESAREC is a Bojac sandy loam (Thermic Typic Hapludults) with 59% sand, 30% silt, and 11% clay with pH ranging from 6.2 to 6.5 and organic matter content of 0.50 to 0.75%. Soil was cultivated to a depth of 30 cm prior for bed preparation. If necessary, overhead sprinkler irrigation was used to bring soil moisture capacity to between 50 and 75% field capacity. Experimental plots were 24 m long with a between row spacing of 1.8 m. Experimental plots were arranged as a randomized complete block design with four replications. The experiment included two drip applied DMDS treatments (374 L/ha (428 kg/ha broadcast) and 468 L/ha (535 kg/ha broadcast)) under TIF and one (561 L/ha (642 kg/ha broadcast)) under VIF as well as one shank treatment (468 L/ha (535 kg/ha broadcast)) under VIF. The fall 2009 experiment did not include the drip applied 374 L/ha under TIF treatment. The shank treatment used a 79:21 w/w formulation of DMDS:chloropicrin (Pic) (United Phosphorus Inc., King of Prussia, PA, USA) that was applied using a single row combination bed press 76 cm wide and 20 cm high with three back swept shanks. Shanks were 20 cm long and fumigant was released at the bottom of the shank. The drip applied treatments used a 79:21 w/w emulsifiable concentrate formulation of DMDS:Pic (United Phosphorus Inc., King of Prussia, PA, USA).

The shank applied fumigant rate was achieved with a Siemens® (Siemens Corporation, New York, NY, USA) flow meter (3.5 L/min of DMDS:Pic 79:21 at 100% flow), TeeJet® (TeeJet Technologies, Wheaton, IL, USA) flow regulators, and tractor speed as described by Gilreath et al. (2005). Bed dimensions were similar between drip and shank treatments. However, the drip treatments contained two drip tapes while the shank treatments contained only one drip tape. Drip applied DMDS was delivered after yellow nutsedge sprouted and started to

emerge through the plastic. Fumigants are more effective when weed germination is initiated and the sprouting seeds or vegetative propagules are exposed to the chemical. Ou et al. (2005) suggest that drip fumigants should be more effective than shank delivered fumigants because the chemical will be dissolved in the water phase and directly contact soil microorganisms which are coated in a layer of water.

Drip applied DMDS was delivered from individual cylinders pressurized with carbon dioxide (similar to the nitrogen pressurized cylinder system described by Ajwa et al., 2002). Known quantities of DMDS were added into the stainless steel spray containers based upon weight. The emulsified fumigant was metered into the drip irrigation with pressure regulators through a manifold which contained a pressure regulator and a back flow preventer. The manifold then branched into three separate lines so all the treatments could be delivered simultaneously. Each line had a valve to control flow rate. Fumigant delivery was monitored as weight loss over time (by using scales to weigh the cylinders) and application rate was adjusted by increasing or decreasing flow rate out of the cylinder. The fumigant was dispersed uniformly with 126,945 L/ha of water delivered by two equally spaced drip tapes (Aqua-Traxx® with the PBX advantage, The TORO Company, El Cajon, CA, USA) operating at a flow rate of 1.7 L/min/30 m over a three hour period. Desaeger et al. (2004) found that drip applied 1,3-D:Pic (60.8:33.3 w/w) delivered through two drip tapes improved water movement and resulted in a more uniform fumigation pattern compared to a single drip tape. In addition, irrigation volumes greater than applied in this experiment resulted in greater lateral movement, but reduced fumigant activity. The system was flushed with water for the last 20 minutes after fumigant application. Beds were formed for all treatments and shank DMDS applications were made on 1 July, 2009, 23 April 2010, 17 June 2010, 26 April 2011, and 23 June 2011. Drip applied DMDS was delivered on 9 July 2009, 3 May 2010, 22 June 2010, 6 May 2011, and 11 July 2011. Drip applications were made 5 to 10 days after the mulch was laid to allow for the nutsedge tubers to sprout. In fall 2011 excessive rainfall (16cm at the ESAREC from bed formation until drip application (ESAREC 2011)) delayed drip application until 18 days after the bed were formed and covered with plastic.

Two mulches were used to compare DMDS retention. During all experiments, either a black (spring) or white on black (fall) formulation of Blockade® VIF (Berry Plastics Corp., Evansville, IN, USA) embossed polyethylene mulch with thickness 0.03 mm containing a nylon barrier was used. Black (spring), or white on black (fall) Vaporsafe® TIF (Raven Industries Inc., Sioux Falls, SD, USA) polyethylene mulch with 0.05 mm thickness containing an EVOH barrier was used. A white on black formulation of TIF was not available during the fall of 2009, so the mulch was painted white the day after application with white exterior latex paint. Comparative temperature readings were taken between the painted mulch and factory white VIF mulch and found to be statistically similar (data not presented). The labeled rate for PaladinEC® under VIF film in tomatoes to control nutsedge is 593 L/ha (679 kg/ha broadcast) and 561 L/ha for shank applied Paladin. DMDS is not currently labeled for use with Vaporsafe® TIF.

Once the fumigant had dissipated, a single row of the tomato cultivar 'BHN 602' (BHNSeed, Immokalee, FL, USA) was transplanted into each plot. Experimental plots contained 25 plants spaced 46 cm apart within the row. Seedlings were transplanted on 7 August 2009, 26 May 2010, 13 July 2010, 31 May 2011, and 3 August 2011. Drip irrigation was provided to meet the water requirements of the crop using a single drip tape. The crop was fertilized based on Cooperative Extension production recommendations (Wilson et al., 2012). Current

recommended cultural and disease management practices for tomato in Virginia were implemented (Wilson et al., 2012).

Data Collection

Weed counts and disease incidence (if present) were recorded at harvest. Tomatoes were harvested at the mature green stage on 3 August 2010, 13 August 2010, 29 September 2010, 7 October 2010, 11 August 2011, 19 August 2011, 27 October 2011, and 14 November 2011. The tomato crop was not harvested fall 2009 due to a late transplant date and severe late blight (*Phytophthora infestans* (Mont.) de Bary) infestation. Harvested tomato fruit were graded and sized according to the USDA grades for fresh tomatoes (USDA, 1991). Yields combined from both harvests for each season are presented.

Data Analysis

Statistical differences in pathogen incidence, nutsedge population, and tomato yields between treatments were determined by analysis of variance (ANOVA). Significant differences between treatment means were separated using Duncan's multiple range test at $P \leq 0.05$.

Results

Fall 2009

Shank applied DMDS under VIF (3 shoots/m²) and drip applied DMDS under both films (0 – 0.09 shoots/m²) controlled yellow nutsedge better than the non-treated VIF (12 shoots/m²) (Table 9.1). Tomato yield data was not collected due to severe late blight infestation that decimated the tomato plants resulting in lowered fruit production and decreased fruit quality.

Spring 2010

All DMDS treatments provided total control of yellow nutsedge (0 shoots/m²), which was better than the non-treated VIF (86 shoots/m²) (Table 9.2). In addition, all the DMDS treatments managed fusarium crown and root rot (*Fusarium oxysporum* f.sp. *radicis-lycopersici* Jarvis and Shoemaker) and southern blight (*Sclerotium rolfsii* Sacc.) (0.8 – 3.3% disease incidence) better than the non-treated VIF (15% disease incidence). There were yield differences in all fruit grade categories between treatments (Table 9.3). DMDS treatments, regardless of rate, mulch type, or application method, produced similar marketable yields (51633 – 56681 kg/ha). The non-treated VIF yielded significantly less marketable fruit (41,387 kg/ha) compared to the DMDS treatments (51633 – 56681 kg/ha). Drip applied DMDS at all rates under both films produced higher extra large fruit yield (34375 – 40019 kg/ha) than the standard rate of shank applied DMDS under VIF (18051 kg/ha). Drip applied DMDS applied at 374 L/ha under TIF yielded higher extra large fruit (40019 kg/ha) compared to the non-treated VIF (26555 kg/ha).

Fall 2010

The non-treated VIF did not control yellow nutsedge (268 shoots/m²), as well as the DMDS treatments (Table 9.4). Nutsedge control was similar between fumigation treatments. There were no yield differences for large and extra large sized fruit. There were yield differences in medium and marketable sized fruit between treatments. All DMDS treatments resulted in greater marketable tomato fruit yields (53766 – 63785 kg/ha) than the non-treated VIF (35100 kg/ha).

Spring 2011

DMDS fumigation at all rates and application methods under both films controlled yellow nutsedge (0.8 – 2.6 shoots/m²) better than the non-treated VIF (235 shoots/m²) (Table 9.5). Nutsedge populations were similar for fumigation treatments. There were no yield differences in any fruit category. Tomato fruit yields were low for all treatments, which might

explain the lack of statistical mean separation between treatments. Tomato transplants were planted late during the spring season into black mulch. High soil temperatures (max air temperature 33.9°C in June (ESAREC, 2011) may have contributed to low tomato fruit yields and explain the results obtained. There was a high yellow nutsedge density in the non-treated VIF, which should have lowered yields compared to the fumigated treatments under normal conditions.

Fall 2011

The non-treated VIF contained more nutsedge (255 shoots/m²) than the DMDS fumigated treatments (5.5 – 116 shoots/m²) (Table 9.6). Nutsedge populations were similar between fumigation treatments. However, nutsedge densities in the fumigated treatments were much higher than in previous seasons. During this season drip applications were not applied until 18 days after bed formation due to rainfall and saturated soil conditions and flooding (the research station received 20.3 cm of rainfall in August 2011 (ESAREC, 2011)). When the fumigant was drip applied there were many nutsedge perforations in the plastic film, which may have resulted in decreased fumigant retention and suboptimal yellow nutsedge control. There were no differences in yield between treatments for any size category. Yields were very low for all treatments. This may be attributed to an unusually wet fall saturated soil conditions during the experiment. In addition, the crop was planted late which exposed the plants to suboptimal growing temperatures and a hard freeze immediately before the second harvest. The non-treated VIF contained a high density of nutsedge which would likely have lowered yields compared to the DMDS fumigated treatments under normal circumstances.

Discussion

Reduced rates of drip applied DMDS under TIF can be used to manage yellow nutsedge and soilborne diseases (fusarium crown rot and southern blight). DMDS has been shown to control yellow nutsedge under LDPE, metalized mulch and VIF (Olson and Rich, 2007). DMDS has also been shown to control *Pythium* spp. (Othman et al., 2010). Resulting pest management from reduced rates of drip applied DMDS under TIF led to increased yields compared to the non-treated VIF. DMDS fumigation has been used effectively to increase yields compared to a non-treated control or maintain yields compared to MBr in tomato (Olson and Rich, 2007; Welker et al., 2006), cantaloupe (Olson and Rich, 2007), and strawberry (Othman et al., 2010; Welker et al., 2007; Lopez-Aranda et al., 2009; Garcia-Mendez et al., 2008). Rates of drip applied 1,3-D plus Pic could be reduced by 33% under TIF compared to HDPE, while maintaining similar weed control (yellow nutsedge, common purslane (*Portulaca oleracea* L.), and common chickweed (*Stellaria media* L.)) and strawberry fruit yield as a standard rate (392 kg/ha) of MBr under HDPE (Fennimore and Ajwa, 2011).

Conclusions

DMDS in an emulsifiable form can be applied through the drip irrigation system to control yellow nutsedge and prevent tomato yield loss. Drip application rates under TIF can be lowered to 374 L/ha, while controlling nutsedge and providing yields similar to DMDS shank applied at 468 L/ha under VIF or drip applied DMDS at 561 L/ha under VIF (20 to 33% reduction in fumigant rate). Grower benefits from drip applying DMDS under TIF are a reduced buffer zone requirement, reduced application rates, and lower worker protection costs because less people would need to be fit tested and trained to wear personal protective equipment.

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Table 9.1. Yellow nutsedge management in a drip applied dimethyl disulfide (DMDS) experiment. Experiments were performed during fall 2009 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA.

Treatment	Nutsedge/m ²
Non-treated VIF	12 a ^z
468 L/ha Drip TIF	0 b
561 L/ha Drip VIF	0.09 b
468 L/ha Shank VIF	3.0 b

^z Means followed by the same letter within a column are not significantly different at $P \leq 0.05$ based on Duncan's multiple range test.

Table 9.2. Yellow nutsedge and disease management in drip applied a dimethyl disulfide (DMDS) experiment. The primary disease agents were fusarium crown rot and southern blight. Experiments were performed during spring 2010 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA.

Treatment	Nutsedge/m ²	% Disease Incidence
Non-treated VIF	86 a ^z	15 a
374 L/ha Drip TIF	0 b	3.3 b
468 L/ha Drip TIF	0 b	0.8 b
561 L/ha Drip VIF	0 b	2.5 b
468 L/ha Shank VIF	0 b	0.8 b

^z Means followed by the same letter within a column are not significantly different at $P \leq 0.05$ based on Duncan's multiple range test.

Table 9.3. Tomato yield in a drip applied dimethyl disulfide (DMDS) experiment. Experiments were performed during spring 2010 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA.

Treatment	Yield (kg/ha)			
	Medium	Large	Extra-Large	Marketable
Non-treated VIF	4825 b ^z	10008 d	26555 bc	41387 b
374 L/ha Drip TIF	3795 b	12868 c	40019 a	56681 a
468 L/ha Drip TIF	4425 b	16018 b	34375 ab	54818 a
561 L/ha Drip VIF	4614 b	15896 b	34700 ab	55210 a
468 L/ha Shank VIF	10130 a	23452 a	18051 c	51633 a

^z Means followed by the same letter within a column are not significantly different at $P \leq 0.05$ based on Duncan's multiple range test.

Table 9.4. Yellow nutsedge management and tomato yield in a drip applied dimethyl disulfide (DMDS) experiment. Experiments were performed during fall 2010 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA.

Treatment	Nutsedge/m ²	Yield(kg/ha)			
		Medium	Large	Extra-Large	Marketable
Non-treated VIF	268 a ^z	5547 c	9793 ns	19759 ns	35100 b
374 L/ha Drip TIF	7.5 b	9005 ab	12448	40345	61797 a
468 L/ha Drip TIF	1.5 b	7390 bc	12612	43782	63785 a
561 L/ha Drip VIF	52 b	8348 ab	11404	34300	54052 a
468 L/ha Shank VIF	32 b	9739 a	10652	33374	53766 a

^z Means followed by the same letter within a column are not significantly different at $P \leq 0.05$ based on Duncan's multiple range test. ns=not significant.

Table 9.5. Yellow nutsedge management and tomato yield in a drip applied dimethyl disulfide (DMDS) experiment. Experiments were performed during spring 2011 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA.

Treatment	Nutsedge/m ²	Yield (kg/ha)			
		Medium	Large	Extra-Large	Marketable
Non-treated VIF	235 a ^z	5665 ns	13789 ns	14338 ns	33792 ns
374 L/ha Drip TIF	0.8 b	7033	14799	14548	36380
468 L/ha Drip TIF	1.7 b	5841	12448	17021	35310
561 L/ha Drip VIF	0.9 b	8490	17807	15009	41306
468 L/ha Shank VIF	2.6 b	6491	15409	16344	38244

^z Means followed by the same letter within a column are not significantly different at $P \leq 0.05$ based on Duncan's multiple range test. ns=not significant.

Table 9.6. Yellow nutsedge management and tomato yield in a drip applied dimethyl disulfide (DMDS) experiment. Experiments were performed during fall 2011 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA.

Treatment	Nutsedge/m ²	Yield (kg/ha)			
		Medium	Large	Extra-Large	Marketable
Non-treated VIF	255 a ^z	3720 ns	5455 ns	3700 ns	12874 ns
374 L/ha Drip TIF	28 b	474	2134	983	3591
468 L/ha Drip TIF	5.5 b	5875	10625	10354	26853
561 L/ha Drip VIF	82 b	6573	11479	6979	25031
468 L/ha Shank VIF	116 b	3964	5895	6485	16344

^z Means followed by the same letter within a column are not significantly different at $P \leq 0.05$ based on Duncan's multiple range test. ns=not significant.

Chapter 10 α -Tomatine and Dehydrotomatine Contents in Leaves and Fruits of Grafted Tomato

Introduction

Glycoalkaloids are N-containing plant secondary metabolites (Friedman, 2002). They are found in many solanaceous plants including eggplant, potato, and tomato. Glycoalkaloid accumulation is generally recognized to protect plants against pathogenic bacteria, fungi, herbivorous insects and animals. Tomatine is a steroidal glycoalkaloid that naturally occurs in tomato. Fruit with high levels of tomatine have a bitter flavor (Rick et al., 1994). Tomatine which was discovered by Fontaine et al. (1948) consists of α -tomatine and dehydrotomatine. Tomatine consists of a hydrophilic group (a tetrasaccharide side chain), a hydrophobic steroidal moiety and a polar secondary NH group. Dehydrotomatine only differs from α -tomatine by the presence of a double bond at C-atoms 5 and 6 of ring B (aglycon tomatidenol). Immature green tomato fruit contain up to 500 mg α -tomatine/kg of fresh fruit (Friedman, 2002). α -Tomatine degrades as the fruit ripens. In mature red tomatoes α -tomatine levels are ~5 mg/kg of fresh fruit weight (Friedman, 2002).

Grafting tomato onto jimson weed (*Datura stramonium* L.) was practiced on a limited scale in the United States to manage root-knot nematodes and was recommended to home gardeners (Kubota et al., 2008). However, grafting tomato to jimson weed was not done commercially possibly because of the potential to transport small amounts of alkaloids to the fruits. The objective of this experiment was to determine if grafting affects tomatine concentrations in foliage and fruit.

Materials and Methods

Plant Material

Leaves, immature green fruit, mature green fruit, and red fruit from a non-grafted commercial tomato cultivar, two non-grafted rootstocks, and tomato grafted on several rootstocks were analyzed for α -tomatine and dehydrotomatine. The plant material analyzed was non-grafted commercial cultivar BHN 602 (BHN Seed, Immokalee, FL), non-grafted rootstock 'Cheong Gang' (Seminis Vegetable Seeds, St. Louis, MO), non-grafted 'Jjak Kkung' (Seminis Vegetable Seeds), 'BHN 602' grafted onto rootstock 'RST 105' (DP seeds, Yuma, AZ), 'BHN 602' grafted on rootstock 'RST 106' (DP seeds), commercial cultivar BHN 602 grafted on rootstock 'Cheong Gang', and 'BHN 602' grafted on 'Jjak Kkung'. Plant and fruit samples were freeze dried before tomatine extraction.

Extraction Process

Tomato glycoalkaloids (α -tomatine and dehydrotomatine) were extracted following a modified protocol adopted from Edwards and Cobb (1996). Pulverized dried plant tissue (0.2 g) was added to 10 mL of extraction buffer (0.02 M heptane sulfonic acid in 1% aqueous acetic acid (v/v)) in a 15 mL centrifuge tube. The contents of the centrifuge tube were mixed by vortexing for 15 s. The contents were then transferred to a 15 mL syringe containing a ¼ of a Kimwipe (Kimberly-Clark Corporation, Neenah, WI, USA) at the tip. The solution was strained through the Kimwipe using vacuum suction to remove large debris. Extracts were then transferred into centrifuge tubes and centrifuged at 4,000 rpm for 15 min to further clarify the solution.

Purification and Concentration

Sep-Pak Classic C18 cartridges were washed with 5 mL of methanol and then 10 mL of extraction buffer. The Sep-pak column was then loaded with the extract. The sample was

treated by removing interfering constituents from the column with a series of washes. The column was washed with 5 mL of water, 5 mL of 0.05 M ammonium bicarbonate, 5 mL methanol -0.025 M ammonium bicarbonate (50:50 v/v), and 5 mL of water. The wash solutions were discarded after passing through the column. The glycoalkaloids were eluted with 1 mL of 80% methanol-0.1% formate. The eluate was collected in an in 1.8 mL Agilent® vials and subjected to HPLC analysis.

Separation and Analysis

The HPLC column was a reversed phase Eclipse® XD5-C18 having dimensions of 2.1 × 100 mm with a particle size of 3.5µm run at room temperature. HPLC solvent A was 10 mM ammonium bicarbonate with 0.00025% formic acid and solvent B was 75% acetonitrile-10 mM ammonium bicarbonate with 0.00025% formic acid. Analysis run time was 32 min, starting with 20 % solvent B from 0 to 0.2 min, then a linear gradient to 36% solvent B at 1 min, 48 % solvent B at 16 min, 100% solvent B at 22 min until 25 min, then decreasing to 20% solvent B from 26 to 32 min. A calibration curve for tomatine was generated by making a dilution series of 0 to 5 µg) of tomatine dissolved in methanol per 20 µL injection volume (0, 0.5, 1, 1.5, 2, 3, 4, and 5 µg tomatine/20 µL methanol). Tomatine amounts were detected with UV absorbance at 202 nm to generate a calibration curve. α-Tomatine and dehydrotomatine peaks were identified based on retention time. After tomatine was detected and quantified a volume of 20 µL of eluate from leaf and green tomato fruit and 40 µL of eluate from red tomato samples from non-grafted and grafted plants were injected into the HPLC. Higher injection volumes were required for tomatine detection in red fruit samples. The dilution series and plant tissue extractions were replicated three times to account for variance.

Data Analysis

Absorbance values from the dilution series was subject to regression analysis to correlate with tomatine amount. Dehydrotomatine and α-tomatine amounts were subject to analysis of variance (ANOVA). Differences between means for tomatine amount for specific tomato plant tissue between plant types were separated using Duncan's multiple range test.

Results

The injected amount of α-tomatine (µg) and responding absorbance values (mAU) followed a strong linear relationship (Figure 10.1). The linear equation was $y = 41.823x - 1.6885$ and had an R^2 value of 0.9742. In addition, dehydrotomatine amounts (µg) and UV absorbance values (mAU) were linearly correlated (Figure 10.2). The linear equation between dehydrotomatine amount and UV absorbance values was $y = 410.52x - 2.516$ and the R^2 value was 0.9947. Dehydrotomatine levels ranged between 192 – 546 mg/kg dry weight in leaves, 5.5 -97 mg/kg dry weight in immature green fruit, 5 – 18.4 in mg/kg dry weight in mature green fruit, and was as high as 6.3 mg/kg in red tomato fruit (Table 10.1). Levels of α-tomatine ranged from 870 – 4308 mg/kg dry weight in leaves, 204 - 1318 mg/kg dry weight in immature green fruit, 171 – 569 mg/kg dry weight in mature green fruit, and 207 – 1715 mg/kg dry weight in red tomato fruit. The percentage of dehydrotomatine (in tomatine) ranged from 11.2 – 21.5% for the leaves, 2.6 – 8.4% in immature green fruit, 1.8 – 4% in the mature green fruit, and 0 (not detectable) – 1.7% in red tomato fruit.

Leaves had the highest tomatine amounts and contained the highest percent of dehydrotomatine. Tomatine levels decreased when fruit ripened from immature green to mature green and the percentage of dehydrotomatine decreased as well. The amount of dehydrotomatine further decreased as mature green fruit ripened to red, which decreased the percent

dehydrotomatine in red fruit. Unexpectedly, based upon the method used, the amount of α -tomatine increased as tomato fruit matured from mature green to ripe.

It appears tomatine levels are not correlated with rootstock type in grafted plants. For example, non-grafted 'Cheong Gang' had different tomatine concentrations than plants of 'BHN 602' grafted on 'Cheong Gang' in leaves, and immature green fruit. In addition, non-grafted 'Jjak Kkung' had different tomatine concentrations compared to 'BHN 602' grafted on 'Jjak Kkung' in immature green tomato fruit.

Discussion

α -Tomatine values were similar to those found in field grown non-transgenic tomato for immature green tomato (Friedman et al., 1994). In addition, the amount of α -tomatine in mature green tomato was lower in this experiment and amounts in red tomato were much higher in this experiment. Friedman and Levin (1995) reported higher α -tomatine values for large immature green fruit and small immature green fruit than observed in these experiments. Large green unripe tomatoes had similar α -tomatine values to this experiment and red fruit had much lower values. Kozukue et al. (2004) also found a higher percentage of dehydrotomatine in leaves, followed by green fruit and undetectable levels in red fruit. In another study, higher percentages of dehydrotomatine were found in small immature green fruit compared to large immature green fruit, however leaf tissue had lower a percentage of dehydrotomatine (Friedman and Levin, 1998). In general, other studies have found that for the tissue analyzed in this study leaves contain the greatest amount of tomatine and tomatine amounts decrease as tomato fruit mature. The current experiment found that same trend with the exception of ripe tomatoes.

Originally, tomatine was not detectable in red fruit when similar injection volumes as extracts of other plant tissues were injected, which is consistent with several other studies. However, increased injection volumes (2x) and increased tissue used for extraction (2x) greatly increased detection levels. It is possible that increased injection volumes and amount of tissue used for extraction does not completely separate from other similar compounds when used with the same HPLC method. Therefore, peak absorbance values may contain contaminants that skewed the results obtained for this experiment.

Based upon the results it appears tomatine is not transported from rootstock to scion. Similarly, Roddick (1982) concluded that steroidal glycoalkaloids from grafted potato and tomato do not appear to transport between rootstock and scion. However, multiple studies show that other types of alkaloids transport between grafted plants (Dawson, 1942; James and Thewlis, 1952; Warren Wilson, 1952a; Warren Wilson, 1952b; Warren Wilson, 1959).

Conclusions

Amounts of α -tomatine, dehydrotomatine and the percentage of dehydrotomatine in tomatine in non-grafted and grafted plants seems to be similar to amounts found in the literature for leaves, immature green, and mature green fruit. The method used in our experiment could not detect tomatine in ripe fruit when the exact same procedures were used as other plant tissues and seems to have greatly overestimated tomatine levels in ripe fruit when injection volumes or plant tissue extracted were doubled. It appears that tomatine levels in grafted plants are not influenced by rootstock. Perhaps further studies could be performed using a complete factorial experiment with multiple scions and multiple rootstocks to confirm that tomatine levels in leaves and fruit of grafted plants are not influenced by the rootstock.

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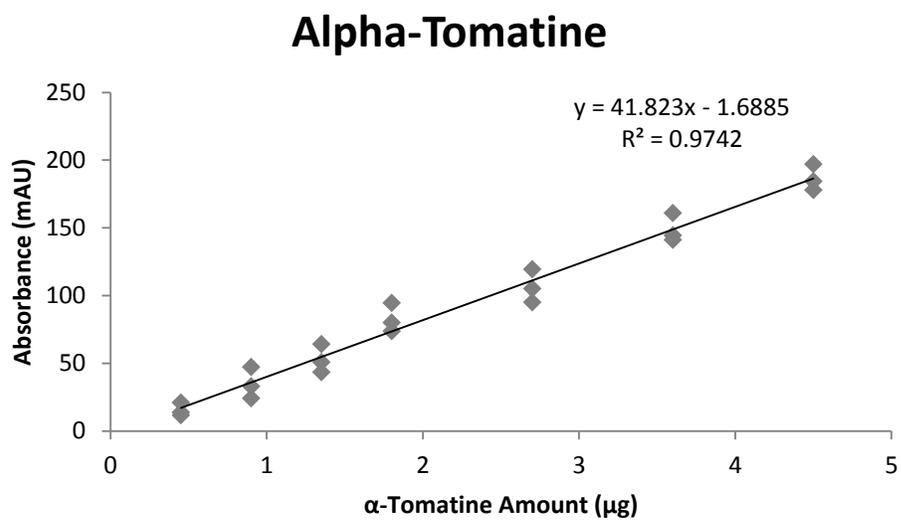


Fig. 10.1. α-Tomatine standard calibration curve from a dilution series subject to HPLC analysis and UV (202 nm) detection.

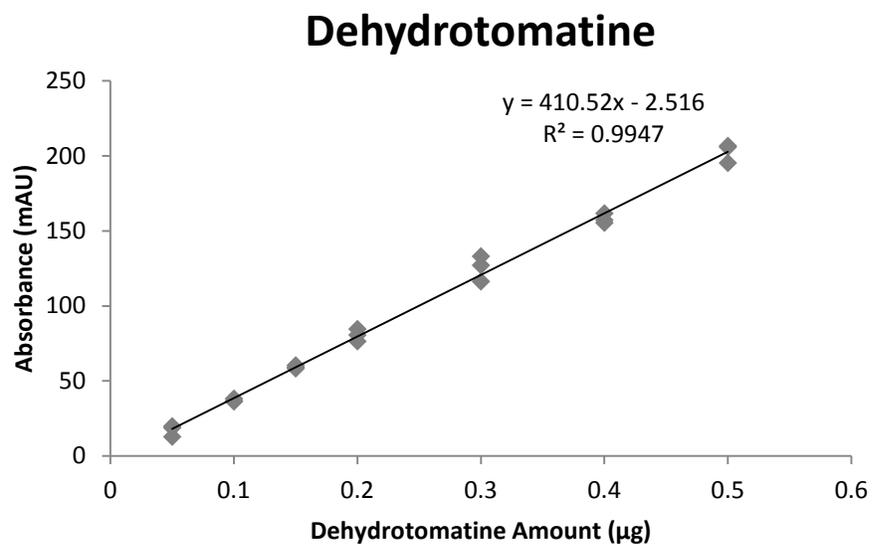


Fig. 10.2. Dehydrotomatine standard calibration curve from a dilution series subject to HPLC analysis and UV (202 nm) detection.

Table 10.1. Amounts of dehydrotomatine, α -tomatine, and the percentage of dehydrotomatine in tomatine for grafted tomato plants and for non-grafted rootstocks or scions.

	Dehydrotomatine (mg/kg dry weight)	α -Tomatine (mg/kg dry weight)	% Dehydrotomatine
Leaves			
Non-grafted BHN 602	423 ab	1599 b	21.0 ab
Cheong Gang	546 a	4308 a	11.2 e
BHN 602/ RST 106	426 ab	1608 b	20.9 ab
BHN 602/RST 105	339 bc	1368 b	19.9 c
BHN 602/Jjak Kkung	244 cd	957 b	20.3 bc
BHN 602/Cheong Gang	192 d	964 b	16.6 d
Jjak Kkung	240 cd	870 b	21.5 a
Immature Green Fruit			
Non-grafted BHN 602	19.5 c	561 bc	3.4 cd
Cheong Gang	97.8 a	1102 ab	8.4 a
BHN 602/ RST 106	37.9 bc	772 bc	4.7 bc
BHN 602/RST 105	5.5 c	204 c	2.6 d
BHN 602/Jjak Kkung	14.7 c	343 c	4.1 bc
BHN 602/Cheong Gang	19.6 c	225 c	8.0 a
Jjak Kkung	68.0 ab	1318 a	4.9 b
Mature Green Fruit			
Non-grafted BHN 602	8.7	424 a	2.0
BHN 602/ RST 106	7.7 ns	171 b	4.0 ns
BHN 602/RST 105	5.0	225 b	2.4
BHN 602/Jjak Kkung	10.5	569 a	1.8
BHN 602/Cheong Gang	18.4	489 a	3.4
Red Fruit			
Non-grafted BHN 602	nd	975 b	0 b
Cheong Gang	6.3	380 c	1.7 a
BHN 602/ RST 106	nd	1715 a	0 b
BHN 602/RST 105	nd	500 bc	0 b
BHN 602/Jjak Kkung	nd	207 c	0 b
BHN 602/Cheong Gang	nd	655 bc	0 b

^z Column means for each plant tissue followed by the same letter are not significantly different at $P \leq 0.05$ based on Duncan's multiple range test. ns = not significant. nd = not detected.