

DEVELOPING ALTERNATIVES TO METHYL BROMIDE:
A FOCUS ON ACROLEIN (2-PROPENAL)

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DEVELOPING ALTERNATIVES TO METHYL BROMIDE:
A FOCUS ON ACROLEIN (2-PROPENAL)

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Lee Julian Simmons

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VITA

Lee Julian Simmons was born February 3, 1977 in Birmingham, Alabama the son of C. Julian Simmons III and Denice Brown Simmons. He has two siblings, Nicholas Grant and Charles Adam Simmons; a wife, Laurel G. Simmons; and a daughter, Kimberly Casey Simmons. In 1995, Lee entered the University of Alabama at Birmingham (UAB) from which he transferred to Auburn University in 1998. In 2000, he received a Bachelor of Science *Magna Cum Laude* in Horticulture. After graduation he was admitted into the Auburn University graduate school and in 2003 completed a Master of Science in Plant Pathology. Continuing at Auburn, Lee was again enrolled in the graduate school in 2003 to earn a Doctorate of Philosophy in Plant Pathology.

DISSERTATION ABSTRACT
DEVELOPING ALTERNATIVES TO METHYL BROMIDE:
A FOCUS ON ACROLEIN (2-PROPENAL)

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Methyl bromide, a soil fumigant with biocidal action, is used in agriculture to control weeds, fungi, bacteria, nematodes, and arthropods. Production of many agricultural commodities including strawberry (*Fragaria × ananassa* Duchesne), tomato (*Solanum lycopersicum* L.), pepper (*Capsicum* spp. L.), and certified sod rely heavily on its usage. Due to methyl bromide's active role in atmospheric ozone depletion, its usage will be phased-out according to the guidelines of the United Nation's Montreal Protocol. Currently, there are no effective alternatives to methyl bromide, leaving these agricultural industries with inadequate means of weed, insect, and pathogen control.

In efforts to develop novel alternatives to methyl-bromide, -enal compounds with pesticidal effects similar to that of methyl-bromide, were tested at Auburn University for efficacy. Several compounds from this group have potential as pesticides, however

acrolein was chosen for further investigation and development. Acrolein is currently labeled as an aquatic herbicide for use in irrigation canals, however little has been done to explore the nematicidal effects of acrolein or to develop strategies and methods to use it in agriculture as an alternative to methyl-bromide. Primary areas of focus in preliminary testing were herbicidal effects on a variety of difficult agronomic weeds and nematicidal effects on two economically important nematodes of Alabama: root-knot nematode (*Meloidogyne* spp. Kofoid and White) and reniform nematode (*Rotylenchulus reniformis* Linford and Oliveira). Greenhouse and microplot studies provided data pertaining to necessary dosimetry and methods of applications required to control plant parasitic nematodes and agricultural weeds.

Herbicidal efficacy: In greenhouse trials, morningglory (*Ipomoea lacunose* L./*I. hederacea* Jacq.), sicklepod (*Senna obtusifolia* (L.) H. S. Irwin and Barneby), jimsonweed (*Datura stramonium* L.), large crabgrass (*Digitaria sanguinalis* (L.) Scop.), and yellow foxtail (*Setaria glauca* L. P. Beauv.) were controlled with acrolein rates ≤ 100 mg/ kg soil, while yellow nutsedge (*Cyperus esculentus* L.) required rates ≥ 250 mg for complete control. Since approximately 150% more acrolein was required to control this species, it was decided to explore combinations of acrolein with yellow nutsedge-specific herbicides in attempts to reduce rates. Combinations of acrolein with halosulfuron, s-metolachlor, EPTC, and propionic acid were successful for reducing rates; however, it was also found that when applied in the same drench, metam sodium and acrolein were antagonistic.

Nematicidal effects: Results from greenhouse trials indicated that drench applications of acrolein at rates 50 to 100 mg/ kg soil effectively controlled the reniform nematode. Control of root-knot by drench application in greenhouse studies required rates of 60 to 200 mg/ kg soil. Acrolein was also effective at controlling stubby-root (*Paratrichodorus minor* (Colbran) Siddiqi) and spiral (*Helicotylenchus dihystra* (Cobb) Sher.) nematodes. Rates required to control plant parasitic nematodes did not drastically affect microbivorous nematode populations.

Soil Enzymatic Activity: In general, as acrolein doses were increased, there was a reduction in soil catalase, α -glucosidase, β -glucosidase, α -galactosidase, β -galactosidase, sulfatase, phosphatase, urease, and chitobiase activity. This is typical of broad-spectrum biocidal activity of a soil fumigant. Acrolein treatments resulted in a slight decrease in soil pH and a slight increase in soil electrical conductivity.

Effects on Fungi, Bacteria, and Actinomycetes: Bacterial colonies decreased as rates of acrolein increased to 200 mg/ kg soil. In contrast, the number of actinomycetes and fungi increased as rates of acrolein increased. This suggests that acrolein is not a biocide; rather, acrolein is a selective pesticide when applied to the soil. Furthermore, while the number of fungal colonies increased, diversity decreased to nearly one fungal genus: *Trichoderma*. As acrolein rates were increased, *Trichoderma* levels greatly increased. This is very significant as *Trichoderma* are desirable soil micro-organisms and can be antagonistic to many plant pathogens. This property of acrolein is not common to soil fumigants.

Conclusions: Acrolein exhibits potential as an alternative to methyl-bromide; however, high rates or combinations with other pesticides are needed to obtain equivalent pest control to that of traditional methyl-bromide/chloropicrin applications.

Style manual or journal used Nematropica.

Computer software used Microsoft Word XP, TableCurve 2D v.5, Statmost32 v.3.5, and SAS v.8

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I. INTRODUCTION

A. METHYL BROMIDE

Methyl bromide is used as a broad-spectrum soil fumigant that is applied prior to planting of commercial agricultural crop to control weeds, fungi, bacteria, nematodes, and arthropods. At the time of application, plastic sheeting is used to cover the soil and retain the chemical to allow fumigant action. Globally, almost 75% of all methyl bromide produced commercially was used for soil fumigation use, with the U.S. being the number one consumer of methyl bromide (Batchelor and Alfarroba, 2002). The prime advantages of methyl bromide are: it has very broad-spectrum control of pest and weeds; it has rapid and consistent action; it is very effective at penetrating soil; it can be used in a wide range of soil types; and, it dissipates quickly after treatment. The disadvantages of methyl bromide are: its high mammalian toxicity and volatility that make handling very dangerous; it reduces soil biodiversity; it has the potential to pollute water and air in neighboring areas; it requires a large amount of plastic sheeting that must be disposed of; it is classified as an ozone-depletor.

In 1974, Molina and Rowland published a study indicating that chlorofluoromethanes could cause the destruction of atmospheric ozone (Nature). Eleven years later, Farman *et al.* reported large losses of the total ozone in Antarctica, a fact that was later commonly-named “The Ozone Hole” (Nature, 1985). In 1991 methyl bromide, i. e. bromomethane, was determined to be one of these ozone-depleting compounds

(Chakrabarti and Bell, 1993). Methyl Bromide was officially added to the list of ozone depleting compounds at the fourth conference of the parties of the Montreal Protocol in 1992. The Montreal Protocol was signed by over 160 countries. This treaty is in place to protect the stratospheric ozone layer, the layer that protects the earth from potentially harmful solar radiation (Batchelor, 1998). The treaty is designed to control and regulate ozone depleting compounds used by mankind. This international agreement called for 25% reduction in methyl bromide (from levels used in 1991) by 1999, 50% reduction by 2001, 70% reduction by 2003 and a complete phase-out by January 2005 for all participating developed nations. Provisions were made for quarantine and critical use exemptions. Developing nations were required to hold consumption in 2002 to their average usage in 1995 - 1998, to reduce usage from this level by 20% in 2005, and to completely phase-out methyl bromide by 2015. In 1993 the United States Environmental Protection Agency announced that methyl bromide was to be phased out by 2001 as ozone depleting compounds fall under their purview (USDA, 2000). This was modified in 1998 and has continued to be altered to suit the political demands of agricultural special interests.

Before the year 2000, it was estimated that approximately 35 million pounds of methyl bromide were used annually for pre-plant fumigation to control weeds and plant pathogenic microorganisms. Tomato acreage accounted for nearly 30% of this total, strawberries for 19%, peppers 14%, various *Prunus* trees and fruits for 16%, and ornamentals and nursery crops for 8% of this methyl bromide usage. California used the most methyl bromide, amounting to 50% of usage in the U.S.A., primarily for their strawberry production (USDA, 2000). Although this number has declined significantly,

it was reported in 1996 that 90% of California strawberries were grown in fields treated with methyl bromide prior to planting. Florida accounted for over 30% of U.S.A. methyl bromide consumption with 90% of their tomato, strawberry, and pepper fields treated in 1996 and 1998 (USDA, 2000).

There are also many post-harvest uses of methyl bromide that are impacted as methyl bromide is being phased out. Shipped and stored agricultural commodities like almonds (*Prunus dulcis* (Mill.) D.A. Webb), figs (*Ficus* spp.), cocoa beans (*Theobroma cacao* L.), dates (*Phoenix dactylifera* L.), raisins (dried *Vitis* spp.), and walnuts (*Juglans* spp.) are often fumigated with methyl bromide to disinfest them of storage pests. Mills, some facilities, warehouses, and even entire cargo ships have been treated with methyl bromide to control domestic and exotic pests. Quarantine uses of methyl bromide will still be allowed. These treatments are found necessary to prevent the spread of potentially devastating pests and diseases (USDA, 2000).

The National Center for Food and Agricultural Policy estimated that the total annual net loss from the phase-out of methyl bromide for pre-plant fumigation would be around \$400 to \$450 million annually. They predicted approximately \$150 to \$200 million would be lost in tomatoes, strawberries, and other vegetable crops; \$140 million would be lost in perennial production; and \$100 million would be lost in ornamentals and nursery crops. With this economic impact, it was predicted that tomato and strawberry production in the U.S.A. would decline while imports from Mexico and other countries would increase. Higher prices in the market due to this reduction in supply were also expected (USDA, 2000), but has yet to happen.

B. ALTERNATIVES TO METHYL BROMIDE

Alternatives to methyl bromide face many challenges. Those chemical alternatives currently registered face regulations restricting their uses due to limits in pollution levels in the air and general air quality concerns. Many growers feel that the efficacy of these registered compounds is less than that of methyl bromide. Township caps, buffer zones, and volatile organic compound (VOC) limits severely restrict the use of alternative compounds. Some of these compounds are limited to certain soil types, regions, and even countries due to groundwater contamination or persistence issues. Those compounds without registration face the same challenges mentioned as well as financial, regulatory, political, marketing, and acceptance hurdles. The time required to obtain registration may limit their uses until after the complete phase-out of methyl bromide has occurred. Currently, there is no universally accepted, EPA-approved methyl bromide alternative. It may be the case that no one alternative compound or method will solely replace methyl bromide. Until suitable and approved methyl bromide alternatives are available, efforts will continue to search for new compounds or methods to control weeds, nematodes, and other soil-borne pests of agriculture.

Alternatives to methyl bromide use in agriculture can easily be divided into (1) non-chemical and (2) chemical means of control.

Non-chemical Alternatives

One of the oldest and most practical means of pest control is through the implementation of cropping systems and crop rotations. Properly developed cropping systems utilize various crops and practices to manage soil ecology for the prevention and

control of agricultural pests (Rodriguez-Kabana *et al.*, 1998). Cropping systems have plasticity and adapt as needed unlike the more strict alternation of crops with rotations. Some rotation systems are ineffective at controlling many pests, while others are very successful. Rotating or alternating between chrysanthemums (*Chrysanthemum* spp.) and carnations (*Dianthus caryophyllus* L.) has been an effective means of dealing with soil-borne pests for Colombian flower producers (Rodriguez-Kabana *et al.* 1998)

Temporarily taking fields out of production is another method used to prevent the build-up of plant pathogens and weeds. However, there is there no yield gathered from fallow fields and many pathogens and weeds can either survive or thrive in fallow situations (Batchelor, 1998).

Composts and organic amendments can also be used to control many soil-borne pests. Large amounts of these are required for needed effects and positive results may take some time before being evident. When managed properly, some composts can create soils that are suppressive to plant diseases (Pereira *et al.*, 1996). Amendments such as chitin have been shown to suppress *Rhizoctonia solani* and nematodes while stimulating beneficial microorganisms (Rodríguez-Kábana, 1998).

Steaming and soil solarization are two methods of soil sterilization and pasteurization. Steam, over 100°C, can be perfused through soil to kill many organisms. The organisms killed and the efficacy of control is dependent on the penetration of the steam into soil, the temperatures reached during steaming, and the duration of steaming (Batchelor, 1998). This is an effective and practical method for treating large volumes of soil used in ornamental greenhouse or nursery production; however, sterilization is expensive and creates microbiological voids in the soil that are often repopulated by plant

pathogens (Runia, 1983). Soil solarization utilizes plastic cover to trap solar radiation and heat to kill soil pests. This method is best suited for hot and arid regions of the world with little cloud cover. In other parts of the world, the only time of the year where the environment is ideal for soil solarization is in the peak of crop production (Batchelor, 1998). It has been used in less than ideal parts of the world (Chellemi *et al.*, 1997); however, results and efficacy are entirely dependent on prevailing weather conditions. Most successful methods of solarization are used as part of an integrated system combined with other pesticides and management practices.

Many different management practices can be used to prevent or control pests. Simple practices such as timing the planting of a crop to avoid pest pressure can be quite successful. Early varieties of soybeans, for example, are used to avoid producing crops when they are most susceptible to asian soybean rust (*Phakopsora pachyrhizi*). Root-knot nematode (*Meloidogyne* spp. Kofoid and White) can be avoided by planting tomatoes at the right time of the year in the Canary Islands (Bello, 1998). Methods of tillage, ploughing, and sanitation can all be used to help control soil-borne pests (Chellemi *et al.*, 1997; Bello, 1998). The use of pathogen-free stock is one of the most important means of preventing disease. This is one of the easiest methods of preventing diseases that are not already present. In situations where pests are already present and problematic, the use of resistant varieties or grafts onto resistant stock is also very effective at reducing or controlling diseases. The use of non-host cover crops and mulches has also been effective at controlling pests (Pizano, 2006). Managing water and moisture levels can be another way to prevent soil-borne pests. Some soil-borne plant pathogenic fungi, like *Pythium* spp., require high soil moisture levels to infect plants.

Preventing high moisture levels is a simple way to prevent diseases caused by these fungi. Another water management practice that can be used to control soil pests is flooding. Rotating soybean (*Glycine max* (L.) Merr.) or cotton (*Gossypium hirsutum* L.) fields to rice (*Oryza sativa* L. and *Oryza glaberrima* Steud.) production, which requires flooding, is an excellent way to reduce plant pathogenic nematodes and fungi (Batchelor, 1998), but is limited due to topography and water requirements.

Mulches, specifically plastic covers used in agriculture, are commonly used and warrant considerable further investigation. They can be used to reduce chemical emissions and in many cases increase effectiveness of compounds used to treat the soil. Two common problems with plastic covers currently used in agriculture are (1) lack of acceptable fumigant/compound retention and (2) sedges, a difficult group of weeds to control that easily compromise the films and may cause breakdown of weed control. Many trials have compared the standard low density polyethylene films (LDPE or PE) and high density polyethylene films (HDPE) for efficacy, and many are now investigating virtually impermeable films (VIF) and most recently metallized films. A study conducted in Florida (Gilreath *et al.*, 2005), compared the use of methyl bromide and chloropicrin (67:33, w:w) under HDPE, VIF, and metallized mulches. They reported much higher concentrations of methyl bromide were retained by the VIF and metallized mulch than the HDPE mulch. They also reported that metallized mulches controlled nutsedge (*Cyperus* spp.) the best, regardless of the treatment rate. Another trial in Florida (Noling *et al.*, 2005), was conducted using previously standard methyl bromide practices (LDPE with methyl bromide 67:33 at 448 kg/ha) and rates reduced by 50 and 75% under VIF. They were able to show that rates of 224 kg/ha under VIF gave the same nutsedge

control as 448 kg/ha under the standard LDPE. It is commonly accepted that plastic mulches can be used to improve efficacy; however, there are substantial costs for using and disposing of the plastic. With some of the more retentive plastic mulches, there are also issues of phytotoxicity and increased plant-back intervals due to increased pesticide retention.

There are a variety of unconventional or novel alternatives to methyl bromide being explored currently. One such “alternative” has been proposed by the Crocker Nuclear Laboratory at the University of California, Davis campus. This group proposes a device that will utilize radiofrequency power (RF) to disinfest field soil. No trials have been conducted nor is there any equipment available to test these theories yet (Lagunas-Solar *et al.*, 2005). Another concept considered to prevent loss of methyl bromide into the atmosphere is the use of scrubbers designed to trap methyl bromide off-gassing (Joyce, 2005).

One of the few biological alternatives to methyl bromide being proposed is *Muscodora albus*, an endophytic fungus that has been isolated from a Honduran cinnamon tree (*Cinnamomum zeylanicum*). In the 2005 Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions, it was reported as effective for control of *Rhizoctonia* sp., *Pythium* spp., *Verticillium* sp., *Fusarium* sp., *Phytophthora* spp., *Sclerotinia* sp., and *Sclerotium* sp. in the greenhouse and effective control of *Meloidogyne* and *Pratylenchus* spp. in the field (Walgenbach, 2005). It was reported that this can also be effectively used in storage container fumigation. It has been suggested

that plant growth promoting rhizobacteria (PGPR), mycorrhizae, and endophytes can be used in integrated pest management strategies (IPM) to replace methyl bromide fumigation (Batchelor, 1998).

Chemical Alternatives

Desirable chemical alternatives to methyl bromide are expected to provide equal or better pesticidal results than methyl bromide, be easy to apply, affordable to use, and cause little or no deleterious environmental impact. An alternative to methyl bromide is also desired to be effective at rates equal to or lower than those used for methyl bromide. Without attaining these expectations, it is unlikely that any alternative will be widely accepted as a replacement for methyl bromide. Methyl bromide is typically applied at 150 - 200 kg/ha (300 - 400 pounds per acre), often in combination with chloropicrin (trichloronitromethane) as 50:50, 67:33, or 98:2 (w/w) combinations. These rates are used to fumigate soil to control all weed, fungal, nematode, and bacterial pest of the soil for the entire crop growing season. Many growers insist that production without methyl bromide is not economically feasible due to pest pressures. Methyl bromide is applied with some type of plastic cover or mulch to reduce emissions into the atmosphere and to increase pesticidal efficacy. Methyl bromide is typically applied via shank injection into the soil; however, some emulsifiable formulations have been developed.

Very few compounds are registered that have broad-spectrum activity, like methyl bromide, and can be used as immediate chemical alternatives. Methyl bromide is a halogenated hydrocarbon, as are the available fumigants 1,3-dichloropropene and chloropicrin. One group of broad-spectrum pesticides, such as metam sodium and

Dazomet, comprise compounds that produce methyl isothiocyanate (MITC) when applied to the soil. The compound iodomethane, another halogenated hydrocarbon, has been recently granted limited registration (Midas®) for soil fumigation. A product containing furfural (Multiguard Protect®) and one containing dimethyl disulfide (Paladin®) also have some limited registration or permit in the United States of America for broad-spectrum control of agricultural pests.

Halogenated Hydrocarbons

This group of compounds has been used historically to fumigate soil for broad-spectrum control of agricultural pests. Methyl bromide belongs to this class of pesticides, as do many other agricultural pesticides like chloropicrin, 1,3-dichloropropene, ethylene dibromide (EDB), and 1,2-dibromo-3-chloropropane (DBCP). DBCP was removed from use in 1978, and EDB in 1981 due to environmental and toxicological considerations. 1,3-dichloropropene was restricted from use due to certain environmental concerns and is still not available for use in some areas and soil types. These compounds volatilize readily and consequently face regulatory scrutiny such as township caps, buffer zones, and volatile organic compound (VOC) emissions regulations. Iodomethane, with limited registration, also belongs to this category of pesticides.

Chloropicrin alone is primarily effective at controlling soil-borne fungal and bacterial pathogens. It is not very effective against weed and nematode pests, and is degraded relatively quickly by soil microorganisms. It is not likely that chloropicrin will be used alone as a methyl bromide alternative; however, it is used in combinations with other pesticides, especially since there are now emulsifiable formulations available (Pizano, M. 2006).

Pesticides consisting of 1,3-dichloropropene or combinations thereof are gaining acceptance as alternatives to methyl bromide in the production of several commodities including strawberries. This compound was used on 20% of the California strawberry crop in 2003 since registration in 2001, and is reported to be used currently in 90% of strawberry acreage (Trout, 2005). The product Inline®, an emulsifiable combination product of 1,3-dichloropropene and chloropicrin, has been used as an alternative to methyl bromide and chloropicrin fumigation. 1,3-dichloropropene is an effective nematicide with limited fungicidal efficacy while chloropicrin provides fungal control with some nematicidal activity. An experiment conducted in Tifton, Georgia compared drip applied Inline® (60.8% 1,3-dichloropropene and 33.3% chloropicrin) at 15, 31, and 46 L/ha (10, 20, and 30 gallons per acre), and Vapam® (42% metam sodium) at 38, 77, and 115 L/ha (25, 50, and 75 gallons per acre). Yellow nutsedge (*Cyperus esculentus*) tubers 10 cm under the drip tape had 69% mortality and only 20% 20.3 cm away from the tape (same depth) with both compounds. Beet (*Beta vulgaris* L.) seeds infested with *Phytophthora capsici* Leonian and *Rhizoctonia solani* Kühn were also placed in the same locations as the nutsedge. Mortality of these pathogens was 87 - 98% under the tape and 28 - 62% 20.3 cm away (Candole *et al.*, 2005). They concluded that these were poor methyl bromide alternatives and required further investigation if they were to be effective. Other researchers, such as Gilreath *et al.* in Florida (2005) and Shem-Tov *et al.* in California (2005) have reported success with Inline® under VIF when it is used as a sequential treatment with metam sodium. This was confirmed in a review of methyl bromide alternatives evaluated by IR-4 (Norton, 2005).

Methyl iodide is very similar to methyl bromide, only with iodine instead of bromine in the structure and has therefore been called a “drop-in-place” alternative to methyl bromide. Iodomethane, or methyl iodide, has a partial pressure curve similar to that of methyl bromide and thus may be a good soil fumigant providing broad-spectrum pest control similar to that of methyl bromide. Due to the heavier molecular weight of iodine, increased active ingredient is required per acre to provide similar control to methyl bromide. Midas® is a mixture of iodomethane and chloropicrin in various ratios. A California study compared Midas® to methyl bromide/chloropicrin combination in strawberry and reported equal or better results for Midas® (Ajwa *et al.*, 2005). A corporate representative for the producers of Midas® reported equal or better results for Midas® containing equal weights of iodomethane and chloropicrin (50:50) compared to methyl bromide in Florida tomatoes (Kreger, 2005). Florida trials in 2006 and 2007 showed that Midas® (50:50) provided similar weed control and tomato yield as methyl bromide (Olson and Kreger, 2007). This compound has been known to cause significant corrosion on storage tanks and application equipment.

Methyl Isothiocyanate Generators

Methyl isothiocyanate (MITC) generators provide broad-spectrum pest control; however, there are concerns and problems with inconsistent results as well as reduction in efficacy with multiple applications. The problems of inconsistency can be attributed to: (a) poor soil distribution of the compound since MITC does not move well through the soil profile and (b) a reduction of efficacy attributed to enhanced microbial degradation. Microbial degradation of many compounds can be accelerated by repeated treatments, and this is a known problem of most MITC generators. Metam sodium (Vapam®),

metam potassium (Kapam®), and dazomet (Basamid®) are all MITC generators applied to the soil prior to crop planting to control a broad-spectrum of agricultural pests. It is also critical that the soil moisture be adequate when applying these compounds as it is the break-down product (MITC) that is active. It is equally critical that the timing of crop-planting be well planned to assure efficacy and to avoid phytotoxicity (Rodriguez-Kabana *et al.*, 1977).

Metam sodium (Vapam®) is a liquid MITC generator used in pre-plant soil treatments. This compound controls some weeds, many fungi, and some plant parasitic nematodes. Proper distribution in the soil can be difficult to achieve, and reduced efficacy over time is problematic due to a build-up of soil microorganisms that can easily degrade the compound and its metabolites (Thomson, 1992). Dazomet (Basamid ®) is a granular pre-plant MITC generator. For effective control using dazomet, good distribution is required as well as mechanical incorporation.

Other Registered Compounds

Dimethyl disulfide (DMDS) is a compound found in nature in wetland areas and in plants like *Allium* spp. and *Brassica* spp. (McKown *et al.*, 2007). DMDS, or Paladin™ is not registered in the U.S.A.; however, this compound was issued an experimental-use permit for May, 2007 to May, 2008 (Robinson *et al.*, 2007). DMDS can be applied on an experimental basis to a maximum of 200 acres of a crop that can not be sold. It is shank injected or drip-applied and can be used alone as well as in combination with chloropicrin. Reports from Florida suggest it may provide weed and soil-borne pathogen control similar to that of methyl bromide (Olson and Rich, 2007). DMDS has also been reported to provide pest control and tomato yields similar to methyl bromide in North

Carolina (Welker et al, 2006; Welker *et al.*, 2007). Results from Alabama indicate DMDS may not be effective at controlling yellow nutsedge when applied alone (Rodríguez-Kábana and Simmons, 2004).

Furfural is a compound found readily in nature and in foods such as bread, baked goods, and coffee; it is produced commercially from the steam-distillation of H₂SO₄-treated sugarcane bagasse (Rodríguez-Kábana, 2006). The fungicidal properties of furfural have been known since the 1920's. Rates required to control difficult agricultural weeds and plant pathogenic nematodes have been studied for the last 10 years (Rodríguez-Kábana, 2005; Rodríguez-Kábana, 2006; Steyn, 2006). Furfural is currently registered for use on 13 crops in South Africa and has some limited U.S. registration for use on non-food greenhouse crops (Burger, 2005). Furfural has been shown to control sting (*Belonolaimus* spp.), lance (*Hoplolaimus galeatus*), ring (*Criconemella xenoplax*), stubby-root (*Paratrichodorus minor*), and root-knot nematodes (Hensley and Myers, 2006). Furfural has been successfully used to control pests of turf in South Africa, and trials are being conducted with turf in the U.S. (Buntting, 2006). High rates of furfural are required to control weeds; it has been suggested that furfural be applied in combination with other pesticides to improve weed control (Rodríguez-Kábana, 2005).

Potential chemical alternatives

There are many compounds that have once been, or currently are being considered for use as alternatives to methyl bromide. Some are progressing toward registration while others are not currently being developed. This can be due to inefficacy, a lack of corporate interest, or due to the enormous expense of pursuing registration.

Ozone has been used as an alternative soil fumigant with sporadic results attributed to poor movement in different soil types (Smith, 1995). Formaldehyde has been used for pre-plant soil treatment; however, results indicated the compound is primarily effective against bacteria (Rodriguez-Kabana *et al.*, 1977). Anhydrous ammonia can be shank applied to the soil without plastic tarping to control a broad-spectrum of pests. High rates of application can cause phytotoxicity in some plants, and the solubility of the compound in water and lack of movement in the soil cause variable results (Rodriguez-Kabana *et al.*, 1981; Rodriguez-Kabana *et al.*, 1982).

Inorganic azides are non-fumigant pesticides with broad-spectrum activity at low rates (Rodriguez-Kabana *et al.*, 1972; Kelley and Rodriguez-Kabana, 1979). A recent study (Shem-Tov *et al.*, 2005) found no significant difference between tomato yields from Inline™ (65% 1,3-dichloropropene and 35% chloropicrin) 150 kg/ha, sodium azide (NaN₃) at 468 L/ha, Midas (50:50 mixture of methyl iodide and chloropicrin) at 100 kg/ha, and chloropicrin at 100 kg/ha as compared to methyl bromide and chloropicrin at 100 kg/ha used under the same type of mulch cover. This study also compared the use of VIF and two different polyethylene films noting no significant difference in yields between them. Walker *et al.* reported in 2005 that SEP-100® (NaN₃ active ingredient) at 113, 141, 168 kg active ingredient per hectare provided commercially acceptable nutsedge control like methyl bromide at 392 kg/ha. In a separate trial, SEP-100® with the post-emergent herbicide halosulfuron provided the best sedge control; methyl bromide at 448 kg/ha provided adequate sedge control, but plant vigor ratings were poor. SEP-100®-treated tomato plots had excellent plant vigor ratings in this same test (Walker *et al.*, 2005).

There are a variety of compounds termed biofumigants. These are compounds added to the soil that either volatilize or release volatile compounds that have pesticidal activity. *Brassica* spp. incorporated into the soil can be used as a biofumigant to control soil-borne pests since many of them contain isothiocyanate producing glucosinolates (Bello, 1998). There are also many essential oils produced by plants that have broad-spectrum pesticidal efficacy, particularly mustard oil which consists primarily of allyl-isothiocyanate (Rodríguez-Kábana and Simmons, 2005).

Recently published work reports acrolein as an effective herbicide able to control plant parasitic nematodes (Rodríguez-Kábana *et al.*, 2003). This compound (Table 1) is being investigated for its potential as an alternative to methyl bromide and is the focus of this Dissertation.

Acrolein as an Alternative

Little information on the use of acrolein as an agricultural pesticide is available; even though acrolein has been used to fumigate soil since at least 1962 (Racuson and Legator, 1962; Kreutzer, 1962; Krenzer, 1971). In 1962 Kreutzer was issued a U.S. patent for rates and methods of application of acrolein to soil for control of plant pathogenic fungi prior to crop planting; included in the patent are reports of pre-emergent weed activity as well as tomato yield increases resulting from acrolein treatments. Racuson and Legator patented a slow release formulation of acrolein used to fumigate the soil for the control of nematodes, bacteria, and fungi (1962). Roe *et al.* patented a method for using acrolein to control biological organisms in bulk storage like methyl bromide has been used for (1995). Werle *et al.* were issued a U.S. patent in 1997 for

methods and compositions to combat microbial, vegetable, and animal pests with acrolein. In 1999 and 2001, Bockowski and Davis were issued U.S. patents for methods to fumigate soil to control nematodes, fungi, bacteria, viruses, and insects. Allan and Schiller patented a method for the subsurface injection of acrolein into soil prior to planting in 2007. In all of these publications, there was no report of specific rates and methods required to control plant parasitic nematodes which are common pests in agriculture. Very little information was available on the rates and methods required to control weeds with acrolein.

A study conducted by McKenry *et al.* (1995) reported acrolein rates used in attempts to control plant parasitic nematodes. This study used acrolein in a test exploring alternatives to methyl bromide in an area where fruit trees were to be replanted. In this plum tree (*Prunus* spp.) re-plant study there was a glyphosate treatment to kill remaining plum trees and their roots, followed by drench treatment of acrolein at 366 kg/ha. This treatment was reported to cause replanted tree growth 8.3 times greater than the nontreated replanted trees: however, it was reported to only give 50% nematode control 1 year after treatment. Other treatments of the study were reported to provide more successful results with 99% nematode control and tree growth increases of 7.0 - 8.5 times that of the nontreated plants. Those treatments were: 1) methyl bromide at 366 kg/ha followed by 18 months of crop rotation, 2) MITC at 732 kg/ha followed by 18 months of crop rotation, 3) glyphosate applied to the site followed by drench application of emulsified 1,3-D at 366 kg/ha, and 4) methyl bromide at 366 kg/ha that was replanted after 6 months (McKenry *et al.*, 1995). The acrolein results were considered unsatisfactory because long-term nematode control for this situation is essential.

C. ACROLEIN:

Acrolein is the simplest unsaturated aldehyde, consisting of three carbons with a very reactive double-bond group and a very reactive conjugate aldehyde group. Acrolein was first isolated by Redtenbacher in 1843 by distillation of fats and glycerin (Sanders, 1958; Eisler, 1994). Acrolein is a clear-yellow liquid with a vapor pressure of 325.7 mm Hg at 30°C; it is classified as a hazardous chemical because it is volatile, flammable, and explosive (Merk, 1989). Acrolein has a very pungent odor known to irritate lachrymatory ducts of the eyes, mucous membranes, and the respiratory tract. Acrolein is readily found in nature, primarily due to combustion of fossil fuels and organic matter; acrolein is also common to cigarette smoke, in the grilling of meats, cooking with oils, dehydration of glycerin, and is a product of the fermentation by several bacteria (Serjak *et al.*, 1953; Soblov and Smiley, 1959; Eisler, 1994; Fullana *et al.*, 2004; Watanabe *et al.* 2006). Acrolein is part of the irritants in smoke that annoys humans. Acrolein is commonly found at 2 - 7 µg/L air in urban cities, at 10,000 µg/L in combustion engine exhausts, and is found at 10,000 - 12,000 µg/L in rooms filled with cigarette smoke. Areas around large fires are known to have the highest levels of acrolein recorded from natural sources. Combustions of woods, such as pine (*Pinus* spp.), can produce 22 - 121 mg acrolein/kg of wood burned. Acrolein is detected in air, water, and even foods like potatoes, onions, coffee, bread, molasses, and raw turkey. The total annual amount of acrolein released into the air is unknown.

Uses

In 1983, approximately 250,000 tons of acrolein was produced for acrylic acid (92%) and methionine production (5%), and for use as an aquatic herbicide (3%). The

ability of acrolein to polymerize in the presence of many different compounds has been exploited in the chemical industry. Polymers formed with acrolein are used in the textile and paper industries, as steel and aluminum coatings, leather tanning, laundry and dish washer detergent production, and photography. Acrolein was used in World War I by the French army in hand grenades (Papite) because of its extremely irritating effects on humans. It has been used since the 1960's as an effective aquatic herbicide, commonly used in large irrigation or drainage canals that can become clogged with difficult to control aquatic weeds. Acrolein is used in the U.S.A., Australia, Argentina, Egypt, Puerto Rico, the Sudan, and other countries throughout the world (Ferguson *et al.*, 1965; Unrau *et al.*, 1965; Bowmer and Smith, 1984). Acrolein is very toxic to aquatic plants; it is reported that most aquatic weeds are killed with acrolein concentrations of 15,000 µg/L water, while most terrestrial field and garden plants can tolerate this level of acrolein (Bartley and Hatstrup 1975; Eisler, 1994; Peterson *et al.*, 1994). These concentrations in water are also high enough to kill fish and other aquatic fauna. Concentrations of 1,500 µg of acrolein/L water are used to control bacteria, fungi, algae, and mollusks in cooling-water systems (Donohue *et al.*, 1966; Rijstenbi and van Galen, 1981). Acrolein is also used to prevent microbial growth in various liquid fuels and to prevent bacteria from clogging large oil pipe-lines.

Persistence and Degradation

Acrolein is stable at pH 6.0 and should be kept away from water and sunlight to prevent polymerization and explosion. The use of hydroquinone and oxygen-free storage help prevent polymerization in industrially produced acrolein. The half-life of acrolein in water is 50 hours at a pH of 6.6, 38 hours at pH 8.3, and less than 20 hours in sea water

(Eisler, 1994). This is due to degradation as well as evaporation. When used as an aquatic herbicide, acrolein degrades rapidly. Factors such as sunlight, amines and alcohols in the water, and reactions with aquatic weeds can all influence and increase the rate of degradation. Ferguson *et al*, (1961) reported that acrolein rapidly degrades in soils regardless of how it is applied; they reported that acrolein degrades rapidly in plant tissue as well. The half-life of acrolein in the atmosphere is only 2 - 3 hours.

Metabolism

The toxic effects of acrolein are credited to its reaction with proteins and sulfhydryl groups. It is highly reactive to thiol groups and rapidly binds with cystine and glutathione. Acrolein is believed to be metabolized into acrylic acid and glyceraldehydes. Acrolein contaminated water is quickly degraded in the upper gastrointestinal tract of mammals due to the low pH. There is a greater risk of inhalation toxicity than by oral intoxication.

Acrolein Toxicity

Aquatic plants- Acrolein has been used to control aquatic weeds since at least 1959 (Overbeek *et al.*). Most submerged aquatic weeds and algae like *Ceratophyllum* spp., *Chara* spp., *Cladophora* spp., *Elodea* spp., *Hydrodictyon* spp., *Hydrilla* sp., *Najas* spp., *Potamogeton* spp., *Spirogyra* spp., and *Zannichellia* spp. are killed by acrolein at concentrations of 1500 - 7500 µg/L water. Floating aquatic weeds such as *Eichornia* spp., *Jussiaea* spp., and *Pistia* spp. require twice as much acrolein to be controlled as submerged weeds (Eisler, 1994; Anderson and Dechoretz, 1982). Anderson *et al.* in 1982 reported acrolein's effective control of *Hydrilla* in irrigation canals and more recently it was reported that acrolein can be used to reduce biomass and seed production

of *Potamogeton pectinatus* L. in irrigation channels (Bentivegna and Fernandez, 2003; Bentivegna *et al.*, 2004). Algal species *Cladophora glomerata* (L.) Kuetzing, *Enteromorpha intestinalis* (L.) Link and Nees and *Anabaena* sp. were killed at very low concentrations of acrolein (Fritz-Sheridan, 1982). Different concentrations at different exposure times can be used to obtain the same herbicidal results. Acrolein treatments in an area of Washington were applied as 0.1 mg acrolein/L water for 48 hours or 1 mg acrolein for 4 hours and achieved the same results. Aquatic vegetation is normally disintegrated 1 week after acrolein treatments, and filamentous algae commonly appear around 1 month after treatment (Eisler, 1994).

Terrestrial plants- Most crops can easily tolerate concentrations of 25,000 µg of acrolein per liter of water. Alfalfa (*Medicago sativa*), castor bean (*Ricinus communis*), corn (*Zea mays*), cotton (*Gossypium hirsutum*), milo (*Sorghum* spp.), squash (*Cucurbita* spp.), sugarcane (*Saccharum officinarum*), and tomato (*Lycopersicon esculentum*) can tolerate 70,000 - 80,000 µg/L water (Ferguson *et al.*, 1961). Acrolein at 500 µg/L of air damaged pinto beans (*Phaseolus* spp.) and morningglory (*Ipomoea* spp.); however, tomato and geraniums (*Geranium* spp.) were not harmed by 1500 µg acrolein/L air (Beauchamp *et al.*, 1985). Pepper plants (*Capsicum* sp.) not only tolerated being watered with acrolein at concentrations used to control aquatic weeds, but showed no residues of the compound a few hours after treatment (Caldironi *et al.*, 2004). Rodriguez-Kabana *et al.* reported of the potential for acrolein to be used to control agricultural weed pests (2003).

Invertebrates- Acrolein has been shown to inhibit DNA, RNA, and general protein synthesis in the bacterium *Escherichia coli* (Migula) Castellani and Chalmers (Beauchamp *et al.*, 1985). The compound caused several different types of cell mutations

in several different genera of bacteria. Some of the metabolites of acrolein are known to cause mutations in bacteria as well as yeasts. Acrolein has also been used to control bacteria in water cooling systems and condensers of industrial plants (Donohue *et al.*, 1966). Benthic invertebrates were killed when acrolein was used in Argentinean irrigation channels (Albarino *et al.*, 2007). Fruit flies (*Drosophila melanogaster*) subjected to acrolein exposure often result in various forms of mutation. The snail pest *Australorbis glabratus*, can be killed when treated with 1,250 to 10,000 µg acrolein/L water for 24 hours, while the American oyster (*Crassostrea virginica*) is killed with only 50 to 55 µg acrolein/L for 96 hours of exposure (Ferguson *et al.*, 1961). Acrolein has been used to prevent mussels from settling and clogging water cooling systems as well (Rijstenbil and van Galen, 1981). Mayflies (*Ephemerella walkeri*) are killed in less than one hour with 100 µg acrolein/L of water while brown shrimp (*Penaeus aztecus*) require exposure for 24 hours for 50% population mortality. Less than 50% mortality was noted when the trematode *Schistosoma mansoni* was exposed to 151 µg acrolein/L for 48 hours. One study tested acrolein for use as an insecticide in grain storage. The vapor pressure curve of acrolein is very similar to that of methyl bromide, suggesting potential for use as a fumigant. However, the study reported that acrolein applied as a liquid to the grain surface and mixed was effective at much lower rates than when acrolein was applied to the headspace of the storage container (Leesch, 1995). Another study reported the ability of acrolein to penetrate stored grains to kill insects; however, the acrolein-treated grains had diminished germination rates (Pourmirza, 2006).

Aquatic Vertebrates- In an EPA report in 1980, acrolein was the most toxic herbicide to fish of the 15 tested. Rainbow trout (*Oncorhynchus mykiss*), small bluegills (*Lepomis*

macrochirus), walleyes (*Stizostedion vitreum vitreum*), and tadpoles have all been accidentally killed during acrolein treatments that were applied to control aquatic weeds (Hill, 1960). Some fish, like bass (*Micropterus* spp.), seem to be able to tolerate herbicidal concentrations of acrolein in water.

Birds- Acrolein was administered to adult mallards (*Anas platyrhynchos*) orally at 9100 µg acrolein per kg of bodyweight, which resulted in confusion, regurgitation, tremors, etc, and finally death. Acrolein at rates as low as 3300 µg caused symptoms of poisoning in the birds. When eggs were treated with acrolein, 51 to 182 µg acrolein was lethal to the embryos (Hudson *et al.*, 1984). Common chicken (*Gallus* sp.) eggs and adults can also be killed with low doses of acrolein (Denine *et al.*, 1971). Chicken embryos that survived acrolein treatment had many various deformities.

Mammals- Acrolein is cytotoxic and ciliostatic in mammals. It is extremely irritating to the eyes and mucous membranes (Eisler, 1994). Acrolein is toxic to mammals through all possible routes of exposure. Oral doses of 4000 µg acrolein per kg of body weight was lethal to guinea pigs (*Cavia* spp.) while mice tolerated diets containing 500 µg acrolein per kg of body weight for over 100 weeks before they began to die (Eisler, 1994). Acrolein has also been used as a pesticide to control mammalian pests such as gophers (Matschke *et al.*, 1998) and ground squirrels (O'Connell *et al.*, 1992). Concentrations of 60,000 µg acrolein per L water resulted in no adverse effects in cows (*Bos* sp.), and rats tolerated 200,000 µg acrolein per L water. Tests with rabbits and guinea pigs showed that acrolein has low dermal toxicity; however, contact with the eyes can cause necrosis (Beauchamp *et al.*, 1985). Due to its high volatility, inhalation toxicity is the greatest danger of acrolein. Humans begin to perceive acrolein in air at

concentrations of 500 - 1000 $\mu\text{g/L}$, and due to its acrid odor, strong lachrymatory effects, and irritation of the respiratory tract, humans naturally attempt to avoid exposure (Beauchamp *et al.*, 1985). Air concentrations of 660 μg acrolein per L killed rats after 24 hours and concentrations of 875,000 $\mu\text{g/L}$ killed mice in one minute (Eisler, 1994). Multiple exposures to low levels of acrolein produced no long-term effects suggesting that toxic effects of acrolein are acute (Eisler, 1994). The human threshold concentration of acrolein for a normal workweek is 110 $\mu\text{g/L}$ air (0.1 ppm) with a short-term exposure limit of 350 $\mu\text{g/L}$ air (0.3 ppm) (Beauchamp *et al.*, 1985). An intake of 47.8 μg of acrolein causes no adverse effects. Gloves, a full-face mask, and protective clothing are required for handling acrolein (Beauchamp *et al.*, 1985; NIOSH, 1990). Acrolein causes particular harm in direct contact of the eyes or respiratory tract, in which case a physician should be seen (Beauchamp *et al.*, 1985).

Chemical Name	2-propenal Alternate names: acraaldehyde, acraldehyde, acrolein, acryladehyde, acrylaldehyde, acrylic aldehyde, allyl aldehyde, aqualin, aquilin, Magnacide H, propenal
CAS Number	107-02-8
Structural formula	CH ₂ =CHCHO
Molecular weight	56.06
Specific Gravity	0.8427 - 0.8442
Color	Clear to yellow liquid
Odor	Extremely irritating and pungent
Boiling Point	52.5 - 53.5°C
Melting Point	-86.95°C
Solubility: Water Organic Solvents	206 - 208 grams/L Miscible
Vapor Pressure	215 - 0220 mm HG at 20 °C
Explosive limits of acrolein vapor	2.8 - 31% in air

Table 1: Nomenclature, chemical properties, and physical characteristics of acrolein (Eisler, 1994).

II. NEMATICIDAL PROPERTIES OF ACROLEIN

A. ABSTRACT

Methyl bromide is a broad-spectrum soil fumigant, relied on by many agricultural industries to disinfest soils of a broad range of pests prior to planting. The phase-out of methyl bromide has prompted many researchers to seek alternative methods of controlling the pests that methyl bromide was used to control. Much of the reliance on methyl bromide soil fumigation is based on the necessity to control plant parasitic nematodes. Due to this, it is crucial that a methyl bromide alternative be able to effectively control plant parasitic nematodes. While the herbicidal properties have been known for over 50 years, the nematicidal properties of acrolein have only recently been reported (Rodríguez-Kábana *et al.*, 2003). Published patents from the 1960's allude to the use of acrolein to control plant parasitic nematodes; however, no specific rates or methods of applications have been reported. More recent work by McKenry *et al.* (1995) along with Rahi and Rich (2003) reported inadequate nematicidal efficacy of acrolein. Experiments focused on two economically important nematodes of Alabama: root-knot nematode and reniform nematode (*Rotylenchulus reniformis* Linford and Oliveira). Greenhouse and microplot studies have provided data pertaining to dosimetry and methods of applications required to control plant parasitic nematodes.

Results from greenhouse trials indicated that drench applications of acrolein at rates 50 to 100 mg/kg soil effectively controlled the reniform nematode. Control of root-knot nematodes in pre-plant samples by drench application required 60 to 200 mg acrolein of greenhouse studies; root-knot nematode populations often rebounded by the end of the experiment. Acrolein treatment also resulted in control of stubby-root and spiral (*Helicotylenchus* spp.) nematodes. Rates of acrolein required to control plant pathogenic nematodes often did not dramatically affect microbivorous nematode populations. In the greenhouse, acrolein proved to be an effective nematicide in soils infested with multiple pest pressures and was effective at controlling plant parasitic nematodes in large volumes of soil. No phytotoxicity was observed in plants grown in acrolein-treated soils, and plants grown in acrolein-treated soils had less nematode damage and were generally larger and healthier than plants grown in non-treated soils. In microplot studies, drench applications of acrolein provided control of root-knot and spiral nematodes comparable to drench applications of metam sodium at 127.8 mg/kg soil (60 gallons per acre or 92 L/ha) 4 months after treatment.

B. INTRODUCTION

Acrolein is a naturally occurring compound produced naturally by the burning of fats through reductive condensation of glycerin. Although it has been known for well over a century, its nematicidal properties have not been studied in detail. There are however indications that the compound could be useful for controlling nematodes. Published work U.S. patents such as No. 3028304 (Kreutzer, 1962), No. 3567776 (Krenzer, 1971), No. 3,052,598 (Racusen and Legator, 1962), No. 5,500,220 (Roe *et al.*,

1996), No. 5866614 (Bockowski and Davis, 1999), No. 6294584 (Bockowski and Davis, 2001), and No. 7255049 (Allan and Schiller, 2007) have all alluded to the potential nematicidal activity of the compound, but none provided clear data on the specific rates and methods of application required to control plant parasitic nematodes. Recent work by McKenry et al. (1995) reported the nematicidal results of acrolein to be insufficient for fumigating prior to *Prunus* orchard replanting. Rhai and Rich tested the nematicidal efficacy of acrolein on root-knot nematode juveniles and they excluded the compound from further testing due to reported phytotoxicity and poor nematicidal efficacy in the greenhouse (2003). This study was conducted to determine the precise dosimetric relationship for this compound on two economically important species of nematodes: root-knot (*Meloidogyne* spp.) and reniform (*Rotylenchulus reniformis*) nematodes. The study consisted of greenhouse and microplots experiments. The greenhouse experiments addressed: (a) the effect of acrolein applied directly to soil and as a drench, (b) acrolein applied to large volumes of soil, (c) the sequential applications of low doses, and (d) the nematicidal performance of acrolein for controlling nematodes in the presence of heavy weed and pest infestations. A microplot experiment compared the performance of acrolein and metam sodium for control of root-knot and spiral nematode in cowpeas (*Vigna unguiculata* (L.) Walp).

C. EXPERIMENTS

Nematode Experiment 1: An experiment with the reniform nematode was conducted to explore the nematicidal activity of acrolein applied directly to soil.

Materials and Methods:

Soil infested with the reniform nematode was collected in Huxford, Alabama (clay loam; pH 6.6; CEC = 4.6 - 9.0 $\text{cmol}_c\text{kg}^{-1}$). Soil was apportioned in 1 kg aliquots into 2 L-capacity bags. A 2.5% stock acrolein solution was prepared by slowly dripping 25 ml of acrolein (99% Aldrich™) into 975 ml of rapidly vortexing demineralized water. Using the stock solution, acrolein at rates: 0, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, and 300 mg/kg soil was delivered directly into the bags of soil. Bags with the treated soil were shaken until the contents were evenly blended and the treated soil was transferred into cylindrical plastic pots (10 cm diameter PVC; 1 mm mesh screen bottom; 110 mm diameter student grade Whatman filter paper placed on screen). The pots were covered with 1 mil low density polyethylene (LDPE) bags secured with rubber bands. Pots were arranged in a randomized complete block design on a greenhouse bench with six replications per treatment. Two weeks after treatment, pots were uncovered and soil samples were taken from each pot by emptying the entire pot into a bag, shaking to evenly mix, and removing a 100 cm^3 soil sample for nematode analysis. The remaining soil was placed back into the pot and five soybean (*Glycine max*) seeds, cultivar “Young”, were planted in each pot. Nematodes were extracted from soil samples using the “salad bowl” incubation technique (Rodríguez-Kábana and Pope, 1981). With this technique soil samples are deposited on a tissue paper placed on a large-mesh container and the container is placed in a salad bowl containing enough water to submerge the sample. Root samples are processed similarly, only the tissue paper is not necessary. Samples are incubated in an air-conditioned room (23°C) for 3 days, when the screens

with samples are removed and the contents of the salad bowl are poured through a 400-mesh screen. Nematodes are retained in the screen and are transferred by washing into a petri dish for identification and enumeration. The soybean plants were grown for 41 days when the experiment was terminated for final nematode sampling and plant assessment. Plants assessments were: number of plants, height of plant shoots (cm), weight of fresh shoots (g), weight of fresh roots (g), and root condition rating (1 - 5; 1= best looking, 5= worst looking root systems).

Data Analyses:

Data were analyzed using SAS software; Fisher's least significant differences (P=0.05) were calculated when F values were significant. Unless otherwise noted, statements made in the results of this experiment and all others in this chapter are for significant differences at P=0.05 level of probability.

Results:

Data from pre-plant samples (14 days after treatment) showed that all acrolein treatments significantly reduced reniform nematode population compared to the controls. Treatments of 100 mg of acrolein/kg soil reduced reniform populations to less than 10 nematodes/100 cm³ soil as compared to the controls with populations in the thousands. No nematodes were detected in pots treated with ≥ 175 mg acrolein (Fig. 1A). Microbivorous nematode populations were affected similarly. There were no significant differences among treatments for soybean germination. In the final sampling, reniform nematodes in acrolein treatments ≥ 50 mg/kg soil were significantly fewer than the control; none were detected in treatments ≥ 175 mg acrolein. Microbivorous nematode populations were increased by the 75 and 100 mg/kg soil rates; however, in all other

treatments they were affected similarly to the reniform populations (Fig. 1B). Reniform and microbivorous nematodes extracted from the roots were significantly increased by the 25 mg rate of acrolein (Fig. 1C). Treatments of acrolein ≥ 50 mg had significantly fewer reniform nematodes in the roots than the controls and roots from treatments with ≥ 175 mg had none. There were no significant differences in microbivorous nematode population from the roots for treatments ≤ 100 mg compared to the controls (Fig. 1C). Shoot height was improved in pots treated with 25 - 150 mg acrolein, while higher rates of acrolein resulted in heights similar to the controls (Fig. 1D). Fresh weight of the shoots followed a similar trend as the shoot heights. With the exception of those in the 300-mg-treated pots, all roots grown in the acrolein-treated pots had better root condition ratings than the controls (Fig. 1E). Soybean root weights were significantly increased in all acrolein-treated pots except those treated with 50, 275, and 300 mg acrolein.

Conclusions:

Results from this trial showed that acrolein can be effective at controlling the reniform nematode and may be useful as an agricultural nematicide. The effective rates of acrolein in this greenhouse test are within levels acceptable for field use. Acrolein was not phytotoxic and actually improved the plants.

Nematode Experiment 2: Since acrolein was able to control the reniform nematode when applied directly to soil, an experiment was conducted to investigate the potential for using acrolein to control root-knot nematode. This experiment also explored the possibility of acrolein applied via drenching in an aqueous solution.

Materials and Methods:

Field soil (pH 5.6, sandy soil, CEC < 4.6 cmol_ckg⁻¹) was collected from a cotton field at the E.V. Smith plant Breeding Unit, in Tallahassee, AL. The soil was from a field referred to as the “cotton wilt patch” that is known to be infested with a variety of weed seeds, seedling pathogens, and plant parasitic nematodes, primarily root-knot nematode. Soil was mixed 50% with sand, deposited into 10 cm diameter PVC pots, and placed on a greenhouse bench. Acrolein treatments were prepared using a 1% stock solution (prepared similarly to Nematode Experiment 1) in a final volume of 100 ml water per pot. Drench applications of acrolein were applied at rates of: 10, 20, 30, 40, 50, 60, 80, 100, 120, 140, 160, and 200 mg/kg soil. Controls received 100 ml water only. Pots were covered with a 2 mil thick high density polyethylene (HDPE) plastic bag immediately following treatment, sealed with a rubber band, and were arranged on the greenhouse bench in a randomized complete block design with seven replications per treatment. Pots were uncovered 10 days after treatment, 100 cm³ soil samples were taken for nematode analysis (salad bowl technique), and 5 “Hutchison” soybean seeds were planted per pot. Soybeans were allowed to grow for 59 days at which point they were taken out of the pots for plant assessments and final nematode sampling. Plant parameters assessed were: number of plants, height of shoots (cm), weight of fresh shoots (g), weight of fresh roots (g), root condition rating (1 - 5; 1= best , 5= worst), and number of root nodules.

Results:

Root-knot populations were reduced in all acrolein-treated pots and were not found in pots treated with ≥ 40 mg (Fig. 2A). Spiral nematodes were significantly reduced by all acrolein treatments and were not detected in pots treated with ≥ 30 mg

(Fig. 2A). Microbivorous nematode populations were also reduced by all acrolein treatment; there were none in the pre-plant soil samples from pots treated with ≥ 80 mg/kg soil (Fig. 2A). Microbivorous populations in the soil rebounded by the final sampling and were stimulated by some treatments (Fig. 2C). There were fewer root-knot nematodes extracted from roots grown in soil treated with ≥ 50 mg acrolein than were found in the controls (Fig. 2B). None were detected in pots treated with ≥ 60 mg (Fig. 2B). Roots grown in any of the acrolein-treated soil had fewer spiral nematodes than the controls. Spiral nematodes were only present in the controls and pots treated with 10 and 40 mg acrolein (Fig. 2B). Populations of microbivorous nematodes extracted from the roots were similar or greater than in the controls; there was stimulation of microbivorous nematodes populations on roots grown in soil treated with 30 mg acrolein (Fig. 2C). No major differences were noted for most plant assessments; however, root condition was improved and the number of (*Rhizobium* or *Bradyrhizobium*) nodules was reduced in pots treated with 50 - 160 mg acrolein per kg soil.

Conclusions:

Although populations were low, acrolein provided good control of both root-knot and spiral nematodes at acceptable rates for field use. Acrolein did not eliminate the microbivorous nematode population, an important beneficial component of the soil biota. Applying acrolein by drench in an aqueous solution also proved to be a viable application method for controlling plant parasitic nematodes.

Nematode Experiment 3: Due to low parasitic nematode populations in the previous experiment, an experiment was initiated similarly to Nematode Experiment 2 to test for

acrolein's potential to be used for control of root-knot nematode with larger populations. Drench application methods were also used as they proved effective in the previous experiment.

Materials and Methods:

The experiment was setup and treated as described in Nematode Experiment 2. Pots were uncovered 10 days after treatment, 100 cm³ soil samples were taken for nematode analysis and five cucumber seeds (*Cucumis sativus* L. 'Marketmore 76') were planted per pot. This host plant is very sensitive to root-knot nematode. Cucumbers were grown for 52 days before being removed from the pots for final soil sampling and plant analyses. Additional plant assessments performed during this experiment included: flower count, open flower count, and seedling height, gall rating, and galls per gram of root. At both soil samplings, 50 cm³ of soil was taken, air-dried, and stored frozen for soil enzymatic analysis.

Results:

Samples taken prior to planting indicated that all rates of acrolein significantly reduced root-knot populations, and rates ≥ 20 mg acrolein practically eliminated the nematode (Fig. 3A). Excellent germination was noted and 3 weeks after planting, cucumber seedlings were significantly taller in all acrolein-treated pots compared to the controls. Cucumbers grown in acrolein-treated pots began flowering earlier than the controls. At the final sampling, fewer root-knot nematodes were extracted from the soil of all treatments > 10 mg/kg soil, and none were detected in pots treated with > 40 mg acrolein. Microbivorous nematode populations in the soil were not eliminated throughout this trial with some treatments having greater populations than the controls (Fig 3B).

Pots treated with > 10 mg acrolein had lower populations of root-knot extracted from the cucumber roots and rates > 60 mg had none (Fig. 3C). Microbivorous populations from the roots were not eliminated by any treatment. The number of cucumber plants and the weight of fresh shoots and roots were significantly greater than the controls for all acrolein treatments (Figs. 3D and 3E). The gall ratings and number of galls per gram of root were significantly lower than the controls for all acrolein treatments (Fig. 3G). Shoot heights in pots treated with ≥ 30 mg acrolein were greater than the controls (Fig. 3D). Root condition ratings were improved for all treatments ≥ 20 mg acrolein (Fig 3E). All acrolein treatments resulted in significantly reduced root galling and number of galls per gram of root; rates ≥ 80 mg acrolein resulted in no galling (Fig. 3F).

Conclusions:

Acrolein provided excellent control of the larger root-knot nematode populations in this experiment. Acrolein showed selection for controlling root-knot nematodes while not drastically affecting microbivorous nematodes. The compound improved all plant growth parameters measured and reduced root galling.

Nematode Experiment 4: Since acrolein provided good control of plant parasitic nematodes in 1 kg pots, an experiment was setup to test the efficacy of the compound on a variety of pests with larger volumes of soil. Larger pots were chosen to allow for the chosen host, individual eggplant (*Solanum melongenum* L.) seedlings to be planted and grown to almost full-size.

Materials and Methods:

The experiment was with large PVC pots (15 cm diameter containing 5.5 kg soil per pot). The pots contained a sand-soil mixture (50:50 v/v) with the previously describe Tallassee, AL field soil. Acrolein at rates 25, 50, 75, 100, and 150 mg/kg soil were drench-applied to pots in a final volume of 1 L using a 2.5% stock solution. Controls were treated with 1 L water only. After treatment with acrolein drenches, pots were covered with LDPE bags and sealed with a rubber band. Pots were arranged on a greenhouse bench in a randomized complete block design with seven replications per treatment. Six days after treatment, a 100 cm³ soil sample was taken from each pot for nematode analysis and one eggplant seedling was transplanted into each pot the following day. Plants were allowed to grow for 65 days before final sampling and termination of the experiment. Plant parameters measured were shoot height, shoot weight, root weight, root condition rating (1 - 5; 1= best, 5= worst), root gall rating (0 - 10; 0= no galls, 10= maximum galling) (Zeck, 1971), and galls per gram of root. Nematodes were extracted from soil and root samples using the “salad bowl” technique.

Results:

Acrolein-treated soil contained fewer root-knot nematode juveniles than the controls; the nematode was detected only in those treated with 100 mg acrolein (Fig. 4A). Microbivorous nematodes were also reduced in all acrolein-treated soil, but they were found in significant numbers in soil samples from all pots (Fig. 4A). There were no significant differences in root-knot or microbivorous populations in any of the treatments by the end of the test (Fig. 4B). Stubby root nematode was detected in the final soil sample and was significantly reduced by the acrolein treatments; none were detected in

the ≥ 100 mg treatments (Fig. 4B). There were no differences in eggplant shoot height or weight among treatments. Root weights were slightly reduced in pots treated with 25, 100, and 150 mg acrolein. No differences were noticed among root condition ratings nor in gall ratings nor in galls per gram of root. Eggplant was an unsuitable host-species in the greenhouse as little plant responses were noted and problematic insect pests were encountered.

Conclusions:

While acrolein did provide good root-knot control after treatment, it was unable to provide control to the end of the test. Results suggest that the highest rate of acrolein could be used to control stubby root nematodes. Results showed again that acrolein did not drastically affect microbivorous nematode populations.

Nematode Experiment 5: Since poor root-knot control was noted in Nematode Experiment 4, a second big-pot test was initiated using tomato (*Lycopersicon esculentum* L.) instead of eggplant to again investigate the pesticidal efficacy of drench applied acrolein on larger volumes of soil.

Materials and Methods:

Pots, soil preparation, and experimental design were as described in Nematode Experiment 4. Acrolein at rates 5, 10, 20, 40, 80, and 120 mg/kg soil were drench-applied to pots in a final volume of 1 L prepared from a 2.5% stock solution. After treatment with acrolein solution or water drenches, pots were covered with polyethylene bags and sealed with a rubber band. Six days after treatment, a 100 cm³ soil sample was taken from each pot for nematode analysis and one 'Rutgers' tomato seedling was

transplanted into each pot the following day. Tomatoes were allowed to grow for 69 days before final sampling and termination of the experiment. Plant growth was assessed as described in Nematode Experiment 4, with the addition of: root gall rating (0 - 10; 0= no galls, 10= maximum galling), and galls per gram of root. Nematodes were extracted from soil and root samples using the salad bowl technique.

Results:

Data from the first soil sampling indicates that all acrolein treatments reduced root-knot nematode populations in the first sampling; rates ≥ 40 mg/kg soil virtually eliminated the nematode (Fig. 5A). Microbivorous populations in pots treated with 5 mg acrolein were significantly greater than in the controls. Microbivorous populations in pots treated with ≥ 40 mg acrolein were lower than in the controls, and there were none in pots treated with ≥ 80 mg acrolein (Fig. 5A). At final sampling, there was a significant increase in root-knot populations as compared to the controls for acrolein rates 5 - 40 mg/kg soil. There were no differences between the controls and the 80 or 120 mg rates (Fig. 5B). There were no differences among treatments at final sampling for microbivorous populations except for the 80 mg rate of acrolein which had larger numbers than the controls. The stubby root nematode was extracted from soil of the controls in the final soil sampling but there were none in any of the soils treated with acrolein. A trend of increasing shoot height with increasing rates of acrolein was apparent (Fig. 5D). The weight of fresh shoots from pots treated with 5 - 20 mg acrolein was lower than the controls; however, those treated with 40 - 120 mg acrolein were significantly heavier than the controls (Fig. 5E). Acrolein treatments of 5 mg/kg soil resulted in heavier fresh root weight than the controls. The 120 mg rate resulted in lower

root weights as compared to the controls. Root condition indices show that roots from the 5 and 20 mg rates had better appearance than the controls, and those corresponding to the 40 - 120 rates were better looking than those from the 5 and 20 mg rates (Fig. 5E). Root-knot indices showed the 5 - 20 mg rates of acrolein resulted in less galling than the controls and roots from the 40 - 120 mg treatments were less galled than those from the 5 - 20 mg rates (Fig. 5F). The 5 and 20 mg rates reduced the number of root galls and the 40 - 120 mg rates resulted in fewer galls than the 5 and 20 mg rates.

Conclusions:

Acrolein provided good pre-plant root-knot control; however, the lower rates of acrolein stimulated the nematode by the end of the test. Microbivorous populations did not show long-term suppression from acrolein treatments. Increasing rates of acrolein improved all plant growth parameters measured except for some root weights.

Nematode Experiment 6: In efforts to reduce the rates of acrolein required to control reniform nematodes, an experiment was conducted to test the efficacy of acrolein applied in sequential low doses to control the nematode.

Materials and Methods:

The experiment was with field soil from Huxford, Alabama (previously described) mixed 50:50 with sand. PVC pots (10 cm diameter) were each filled with 1 kg of soil-sand mixture and placed in a greenhouse. Acrolein was applied by drench application at rates: 5, 10, 20, 30, 40, and 50 mg/kg soil in water to a final volume of 100 ml. Applications were made three times with 7 days between each application. Controls received 100 ml water only. Pots were covered after the first treatment with HDPE

plastic bags and sealed with rubber bands. Pots were uncovered to apply the second and third treatments, and were recovered after treatments with HDPE bags and resealed with rubber bands. Pots were uncovered for the second and third treatment and finally 1 week after the third treatment. One week after the final applications, a 100 cm³ soil sample was taken from each pot for nematode analysis using the salad bowl extraction technique. After sampling, five cotton seeds were planted in each pot and allowed to grow for 52 days before the plants were removed from the pots for final soil sampling and plant assessment. Plant parameters assessed were: number of plants, height of shoots (cm), weight of fresh shoots (g), weight of fresh roots (g), and a root condition rating (1 - 5; 1= best , 5= worst).

Results:

There were fewer reniform nematodes in samples from all pots treated with acrolein than in the control pots in the pre-planting soil sample (Fig. 6A). Microbivorous nematodes were not significantly affected by any acrolein treatment (Figs. 6A). By the end of the test, there were no significant differences in reniform or microbivorous populations in the soil or root samples (Figs. 6B and 6C). There were no differences in the number of surviving plants among treatments (Fig. 6D). Soil treated with the 10, 20, and 30 mg rates of acrolein had taller shoots than the controls, and those treated with 30 and 50 mg acrolein had heavier shoots (Fig 6D). There were no differences in root weights among treatments; however, roots from soils treated with ≥ 30 mg acrolein had improved root condition ratings (Fig. 6D).

Conclusions:

Sequential treatments of low rates of acrolein were able to control reniform in the pre-planting samples but did not provide control throughout the experiment. These treatments did result in some plant improvements but with no clear dose response.

Nematode Experiment 7: A series of experiments were conducted to explore the effects of acrolein on soil microorganisms. This experiment was initiated to investigate the effects of acrolein on nematodes and soil microorganisms. The microbiology data will be presented in Chapter IV.

Materials and Methods:

Soil for the experiment was from a Tallassee, Alabama cotton-field that is problematic with fungal seedling diseases and plant parasitic nematodes. Soil was mixed 50:50 (v/v) with sand, apportioned as previously described 1 kg pots, and placed on a greenhouse bench. Acrolein was delivered in 100 ml final volume per pot at rates: 20, 40, 60, 100, 140, 160, and 200 mg/kg soil using a stock 1% solution. A water-treated control was included. After treatment pots were covered with plastic bags, sealed with rubber bands, and were arranged on a bench in a randomized complete block design with seven replications per treatment. Pots were uncovered 10 days after treatment and 50 cm³ soil samples were taken from each for microbial analysis (see details in Chapter 4). After the soil sample was taken, five 'Marketmore 76' cucumber seeds were planted per pot and the pots were replaced in the greenhouse. Cucumbers were allowed to grow for 51 days at which time the test was taken down for final plant growth and nematode analyses. Nematodes were extracted from the cucumber roots and from 100 cm³ final soil

samples. Plant parameters assessed were: number of plants, height of shoots (cm), weight of fresh shoots (g), weight of fresh roots (g), root condition rating (1 - 5; 1= best, 5= worst), and gall rating (0 - 10; 0= no galling, 10= maximum galling).

Results:

Only the 200 mg rate of acrolein significantly reduced root-knot nematode by the end of the test (Fig. 7A). There were no differences in microbivorous populations amongst treatments (Fig. 7A). All acrolein treatments resulted in fewer spiral nematodes in the soil samples than the controls (Fig. 7A). The number of root-knot nematode juveniles extracted from cucumber roots grown in soil treated with the 40 mg acrolein rate was higher than in roots from the controls; all other treatments were statistically similar to the controls (Fig. 7B). Only the 200 mg acrolein treatments resulted in roots with fewer spiral nematodes than the controls (Fig. 7B). There were no statistical differences in number of microbivorous nematodes from the roots of any treatment (Fig. 7B). There were no significant differences in the plant parameters assessed.

Conclusions:

The highest rates of acrolein used were unable to provide complete control of spiral nematodes, although increasing rates of acrolein did reduce the nematode populations. Acrolein treatments did not control root-knot nematode to the end of the experiment.

Nematode Experiment 8: An alternative to methyl bromide must provide broad-spectrum control, so an experiment was conducted to test the efficacy of acrolein in

extreme pest conditions, *i.e.*, soil infested with severe levels of weeds, plant parasitic nematodes, and plant pathogenic fungi.

Materials and Methods:

Soil for the experiment was a 50:50 (v/v) mixture of sand and soil from Tallassee, AL. The soil was from a field with significant levels of weeds, plant parasitic nematode, and plant pathogenic fungi. The soil mixture apportioned in 1 kg amounts in pots as per Nematode Experiment 1. Pots were arranged in a greenhouse in a randomized complete block design with seven replications per treatment. Acrolein at rates: 40, 80, 120, 160, 200, 240, 280, 320, 360, 400, 440, and 480 mg/kg soil were drench applied in final volumes of 100 ml solution per pot, and controls were treated with 100 ml water only. Drench solutions were made using a stock 2% acrolein solution prepared immediately before treatment. Pots were covered with HDPE plastic bags and sealed with rubber bands immediately following treatment. The plastic bags were removed 1 week after treatments and weed counts were taken at 7, 13, and 20 days after treatment. The weeds were then removed, a 100 cm³ soil sample was taken for nematode analysis (salad bowl technique), and each pot was planted with 5 “Hutcheson” soybean seeds. The soybeans were allowed to grow for 6 weeks before they were removed from the pots for plant growth assessments and final nematode sampling. Plant parameters determined were: number of plants, height of shoots (cm), weight of fresh shoots (g), weight of fresh roots (g), and a root condition rating (1 - 5; 1= best, 5= worst).

Results:

Microbivorous nematode populations were initially suppressed by acrolein treatments (Fig. 8A); however, these populations rebounded by the final soil sampling

and were significantly increased in the 40, 80, and 120 mg/kg soil rates (Fig. 8B). Stubby root nematode was the only plant parasitic nematode found in the experiment and it was completely controlled by all acrolein treatments (Fig. 8C). The number of soybean plants was highest and tallest in the acrolein-treated pots (Fig. 8D). The weights of fresh roots and shoots from the acrolein-treated pots were also greater than the controls (Fig. 8E). Root indices were significantly improved by acrolein treatments (Fig. 8F). Weed data will be presented in Chapter III.

Conclusions:

Acrolein provided excellent control of stubby root nematode. Acrolein treatments resulted in excellent plant improvement.

Nematode Experiment 9: Since all rates completely controlled stubby root nematodes in the previous experiment, a similar test was conducted using lower rates of acrolein to determine the minimum rates required to control the nematode.

Materials and Methods:

The experiment was conducted as described in Nematode Experiment 8 except the acrolein treatments were lowered to 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 mg/kg soil; the final solutions were prepared from a stock 0.25% acrolein solution. All assessments and analysis remained the same as the previous experiment except weed counts were conducted 7, 15, and 21 days after treatment.

Results:

Stubby root nematode was controlled in all pots treated with > 5 mg acrolein/kg soil (Fig 9A). Microbivorous nematode populations remained intact for all treatments

(Fig. 9B). Germination and plant growth were improved in soil treated with acrolein as the number of plants, weight of shoots, height of shoots, and the weight of roots were greater in most acrolein-treated pots than in the controls (Figs. 9C and 9D). Roots of plants grown in acrolein-treated soil were noticeably healthier looking than those from control soil (Fig. 9E).

Conclusions:

Low rates of acrolein will control stubby root nematode. All rates of acrolein used in this experiment tended to improve plant growth and appearance.

Nematode Experiment 10: Since most of the greenhouse results were positive, an experiment using drench applications of acrolein was conducted in microplots. Microplot experiments allow testing of pesticidal efficacy in a situation approaching field conditions.

Materials and Methods:

Microplots consisted of terra cotta flu pipe, 0.6 m long (buried), with an approximately 0.3 m by 0.3 m square opening at the bottom and top. These plots contain soils infested with weeds, nematodes, and other pathogens. Acrolein was drench-applied at 50, 75, 100, 125, and 150 mg/kg soil in 3.785 L final volume. Metam sodium (Vapam®), a common soil fumigant, was applied at the recommended rate of 60 gallons per acre (92 L/ha or 127.8 mg/kg soil) and a water-treated control was included. The experiment was setup using a randomized complete block design with eight replications per treatment. Plots were covered with plastic bags and sealed with large rubber bands. After 5 days, plots were uncovered and weed survival ratings were made from digital

photographs taken at 5, 8, and 17 days after treatment (rating scale 0 - 5; 5= no weed control, 0= complete weed control). Cowpea seeds were planted five per plot 20 days after treatment, and one papaya (*Carica papaya* L.) seedling was planted in each plot 34 days after treatment. Soil samples were taken 119 days after treatment for nematode analysis. The samples were taken to a depth of 10 to 15 cm from the root zones of the plants with a 2.5 cm diameter soil probe. Five probes were taken from each plot for the sample. Samples were deposited into labeled plastic bags, stored in the shade, and taken to the lab within an hour of being taken. Each sample was homogenized in the lab and they were setup for nematode extraction using the salad-bowl technique.

Results:

Herbicidal activity will be discussed in Chapter III. All plots treated with acrolein had fewer nematodes than the controls 119 days after treatment (Fig. 10A). Nematodes were detected in plots treated with 50 and 75 mg acrolein but the population counts were not statistically different from those plots where none were detected (Fig. 10A). The metam sodium treatment resulted in more root-knot and spiral nematodes than all other treatments (Fig. 10A). Plots treated with ≥ 125 mg acrolein and the metam sodium treatment had more microbivorous nematodes than the controls (Fig. 10B). Plots treated with 125 mg acrolein had more dorylaimoid nematodes than the controls (Fig. 10B).

Conclusions:

Acrolein provided superior control of root-knot and spiral nematodes compared to the metam sodium treatment; this effect was still evident 119 days after treatment. Increasing rates of acrolein increased the number of microbivorous nematodes in the microplots.

D. DISCUSSION

Direct applications of acrolein to infested soil resulted in excellent control of the reniform nematode with complete control at rates of 75 - 100 mg/kg soil; these rates did not drastically affect microbivorous nematode populations. The same rates of acrolein resulted in healthier and larger plants suggesting that acrolein may be useful to disinfest agricultural fields prior to planting like methyl bromide. These acrolein rates are equivalent to 75 - 100 kg of acrolein per hectare (150 - 200 lbs/acre) which is less than the 175 - 225 kg of methyl bromide normally used to treat a hectare (350 - 450 lbs/acre), therefore, these rates of acrolein are within an acceptable range for agricultural use. Acrolein is soluble in water which allows it to also be applied in aqueous solutions, such as drip and drench applications. The greenhouse studies indicated that drench applications of acrolein controlled the root-knot nematode, the stubby-root nematode, and the spiral nematode at 100 - 125 mg while microbivorous nematodes were normally found in significant numbers in pots treated at these rates. In some tests, acrolein treatments did not control root-knot nematode through the end of the experiment, indicating that acrolein treatments may be most effective against this nematode if they are applied in combination with other nematicides or broad-spectrum pesticides. A series of low-rate drench applications of acrolein can be used to reduce nematode populations using less acrolein; however, these treatments also did not result in nematode control to the end of the test. These rates of acrolein also do not result in the plant improvements observed with higher rates of acrolein in other experiments. The microplot experiment showed that acrolein can be an effective nematicide when applied as a drench. Root-knot and spiral nematodes were completely controlled by 100 kg/ha rate of acrolein 4 months

after treatment while metam sodium at 127.8 kg/ha (127.8 mg/kg) soil stimulated populations of the nematodes. It is very significant that acrolein was more effective at 100 mg/kg soil than a registered, broad-spectrum pesticide at like metam sodium at 127.8 kg/ha. Acrolein may be an effective pesticide, able to control plant pathogenic nematodes in agricultural fields prior to planting, and can be applied by either direct application (shank injection), or in aqueous solution (drip and drench application). Effective rates have been shown to result in better and healthier plants, which is essential for agricultural use.

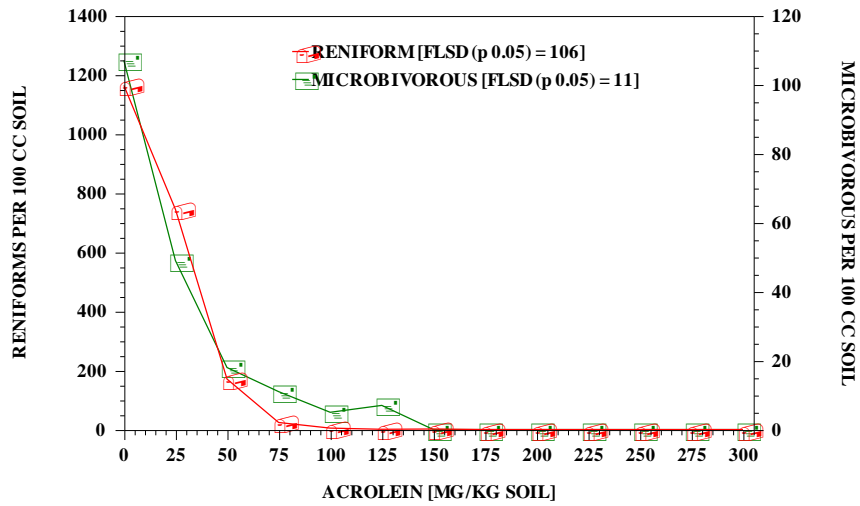


Figure 1A: Reniform and microbivorous nematodes from pre-plant soil sample (Nematode Experiment 1)

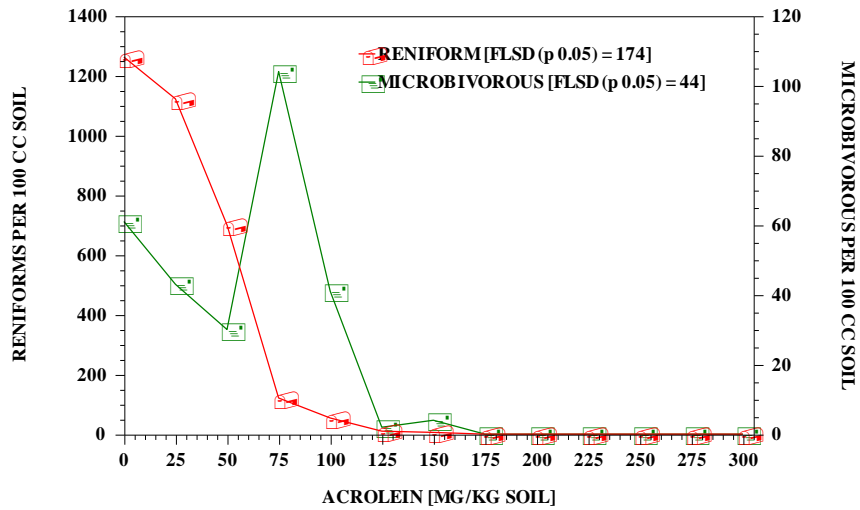


Figure 1B: Reniform and microbivorous nematodes from final soil sample (Nematode Experiment 1).

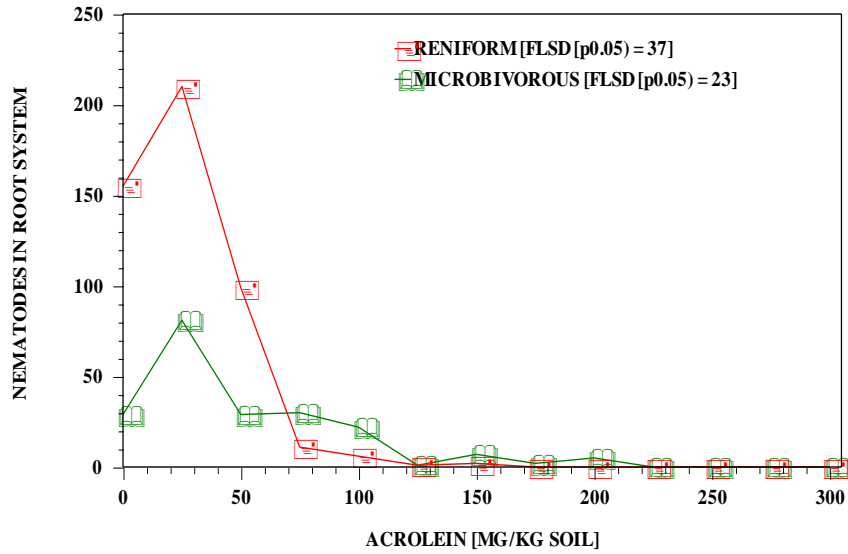


Figure 1C: Reniform and microbivorous nematodes from soybean roots (Nematode Experiment 1).

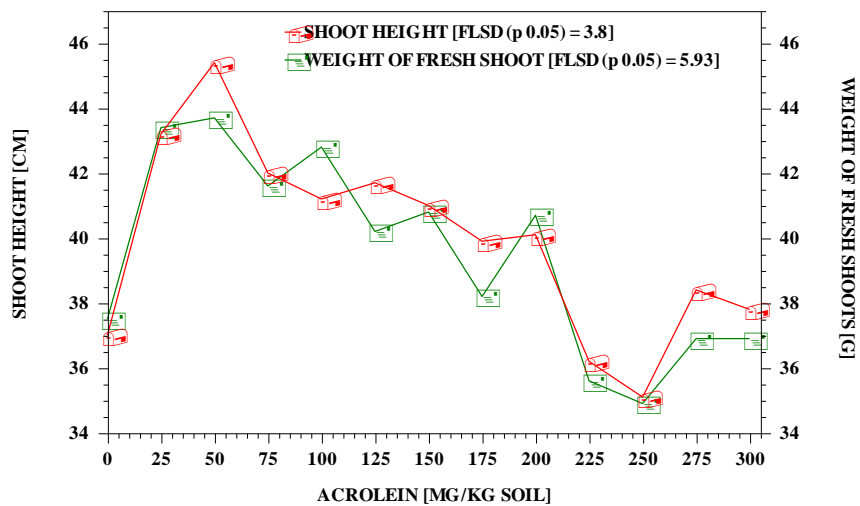


Figure 1D: Height of soybean shoots and weight of fresh roots (Nematode Experiment 1).



Figure 1E: Weight of fresh soybean roots and root condition rating (Nematode Experiment 1).

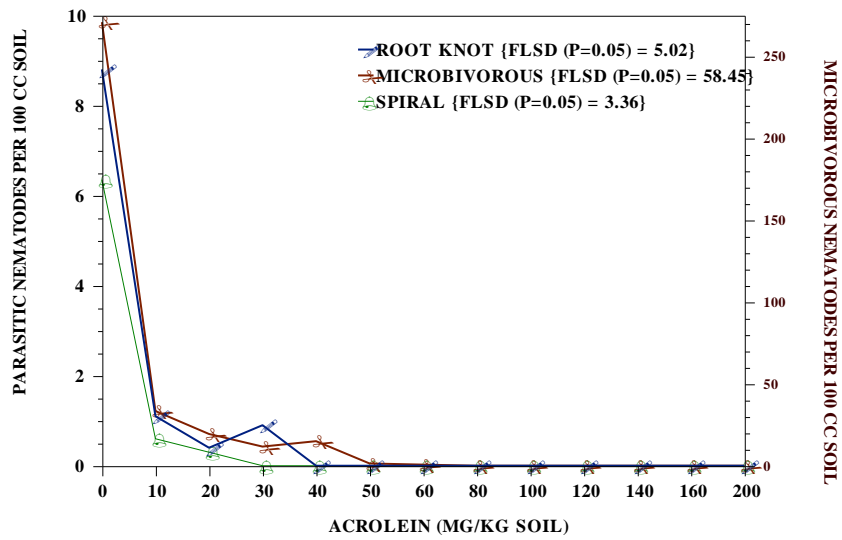


Figure 2A: Root knot, microbivorous, and spiral nematodes from pre-plant sample (Nematode Experiment 2).

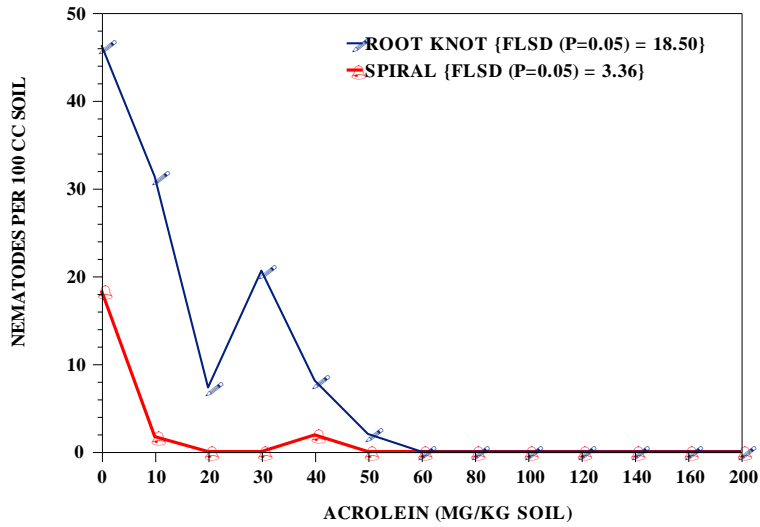


Figure 2B: Root knot and spiral nematodes from final soil sample (Nematode Experiment 2).

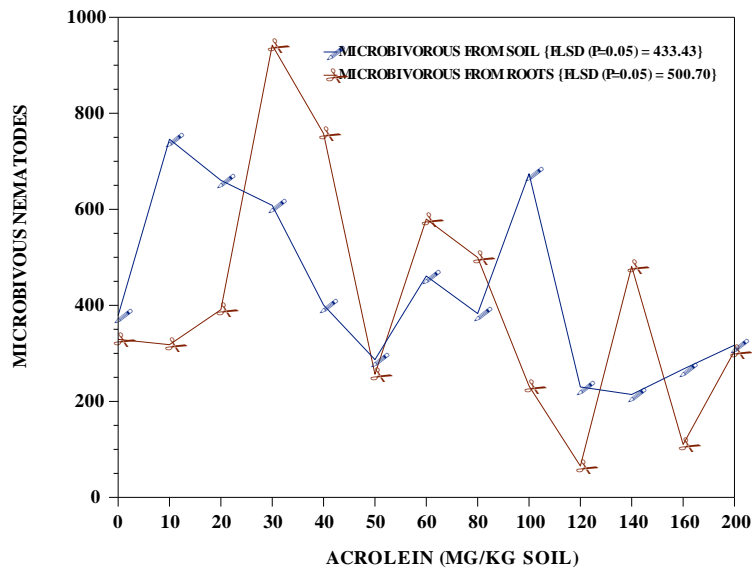


Figure 2C: Microbivorous nematodes from cucumber roots and from final soil sample (Nematode Experiment 2).

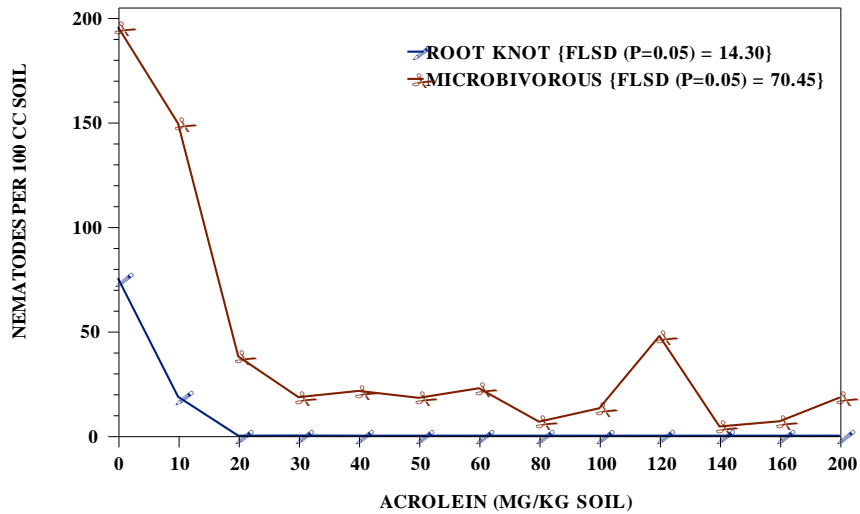


Figure 3A: Root knot and microbivorous nematodes from pre-plant soil sample (Nematode Experiment 3).

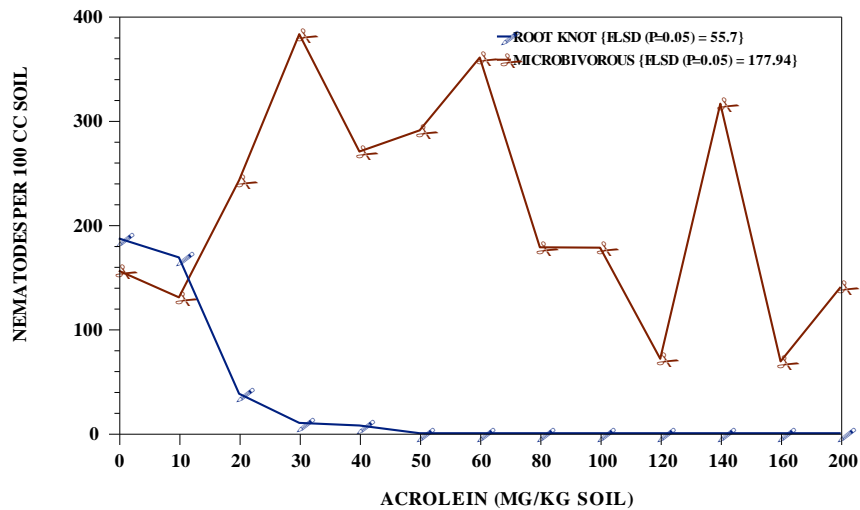


Figure 3B: Root knot and microbivorous nematodes per 100 cc soil at final sampling (Nematode Experiment 3).

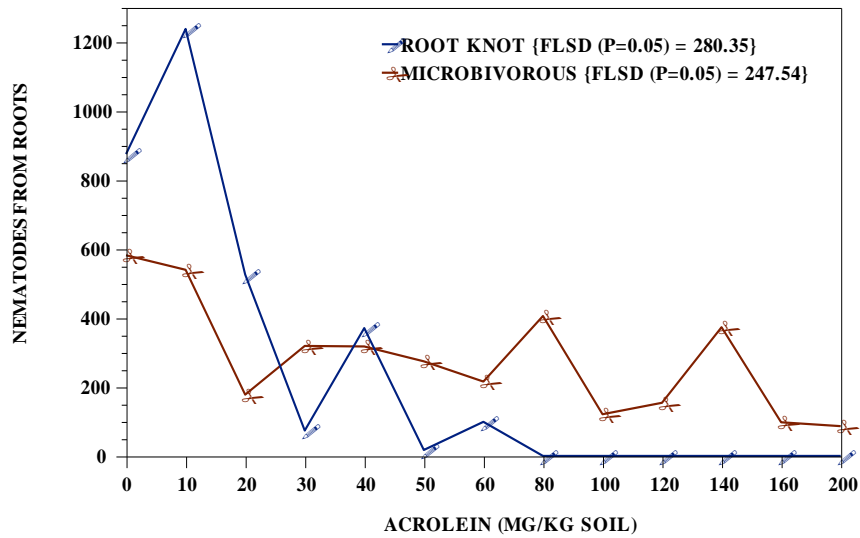


Figure 3C: Root knot and microbivorous populations from cucumber roots (Nematode Experiment 3).

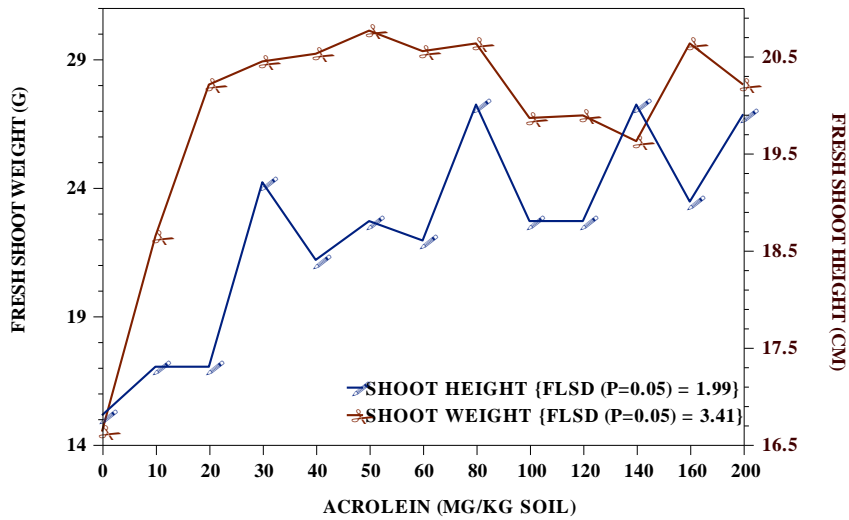
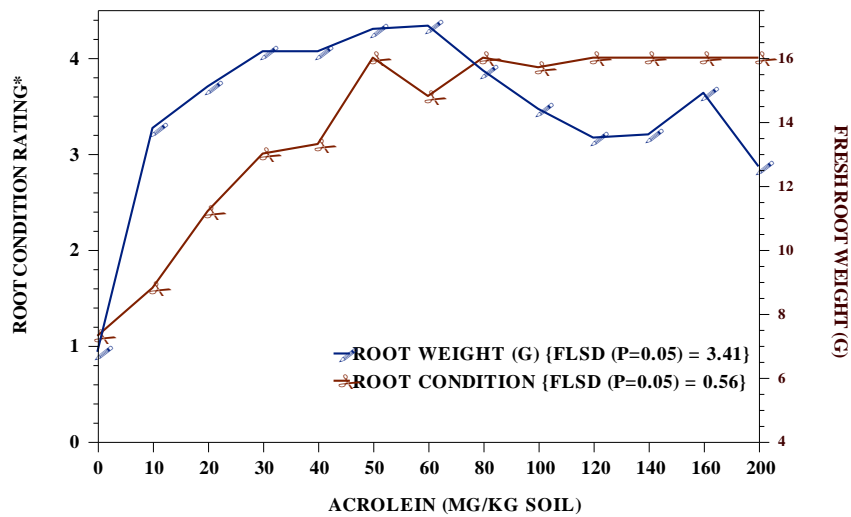
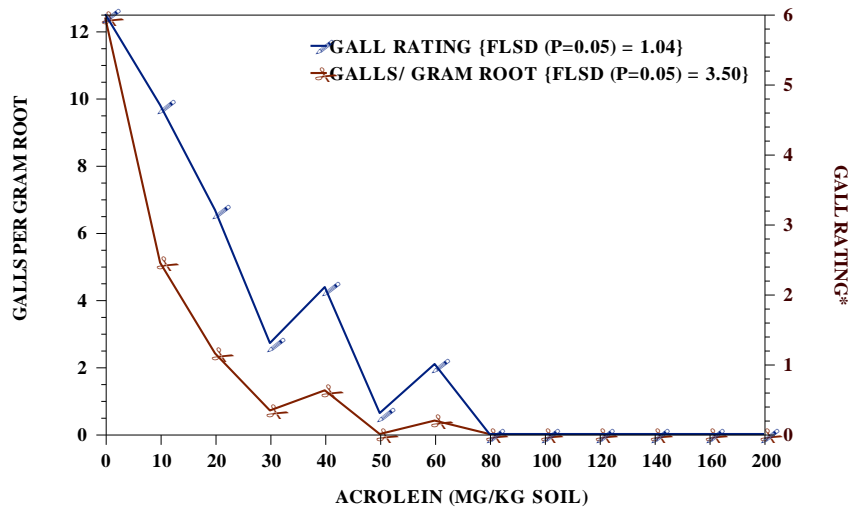


Figure 3D: Fresh cucumber shoot heights (cm) and weights (g) (Nematode Experiment 3).



*ROOT CONDITION INDEX SCALE: 1= BEST TO 5= WORST

Figure 3E: Cucumber fresh root weight (g) and root condition rating (Nematode Experiment 3).



*ROOT-KNOT INDEX SCALE: 0= NO GALLS TO 10 MAXIMUM GALLING

Figure 3F: Cucumber root gall rating and galls per gram of root (Nematode Experiment 3).

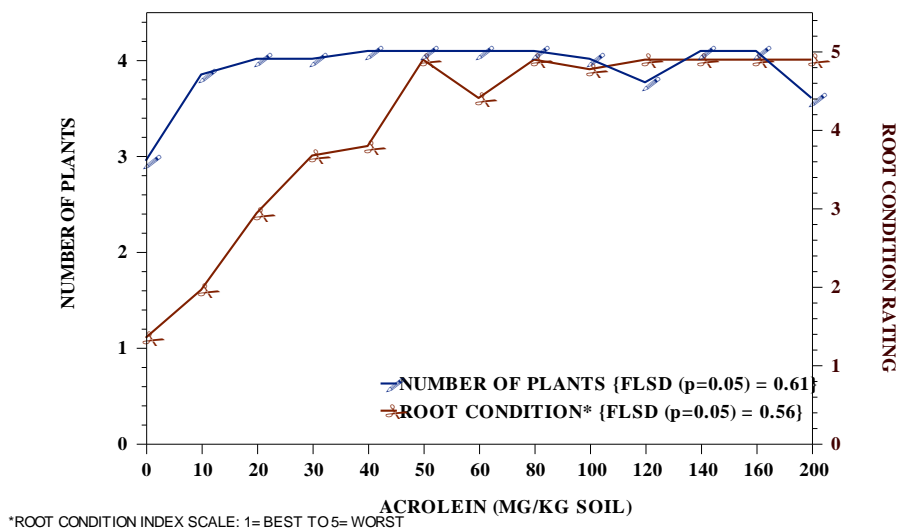


Figure 3G: Number of cucumber plants and root condition rating (Nematode Experiment 3).

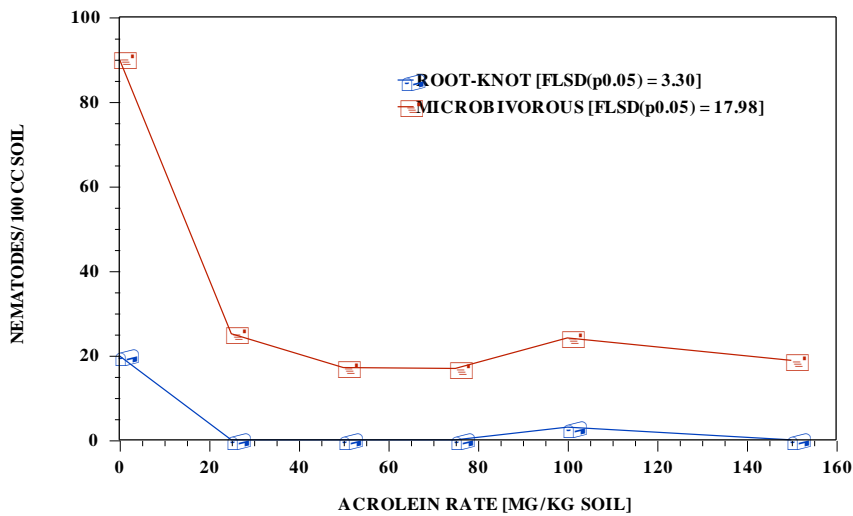


Figure 4A: Root-knot and microbivorous nematodes from the pre-plant soil sample (Nematode Experiment 4).

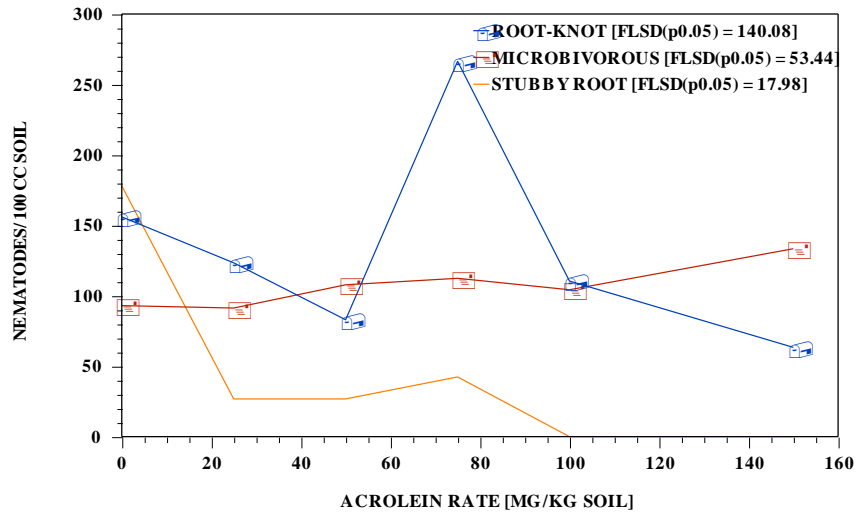


Figure 4B: Root-knot, stubby root, and microbivorous nematodes from the pre-plant soil sample (Nematode Experiment 4).

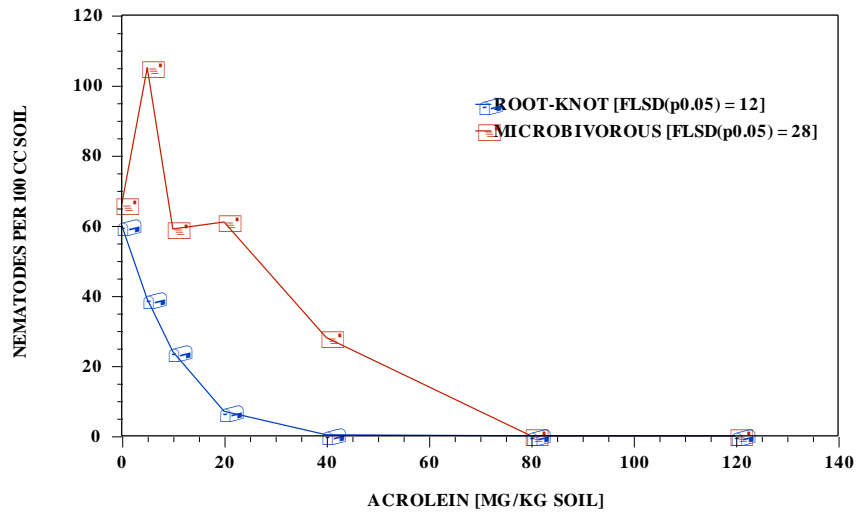


Figure 5A: Root-knot and microbivorous nematodes from pre-plant soil sample (Nematode Experiment 5).

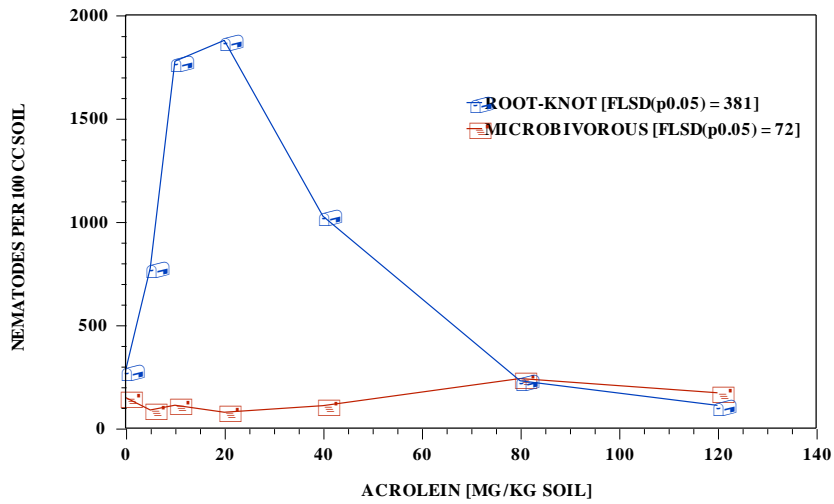


Figure 5B: Root-knot and microbivorous nematodes from final soil sample (Nematode Experiment 5).

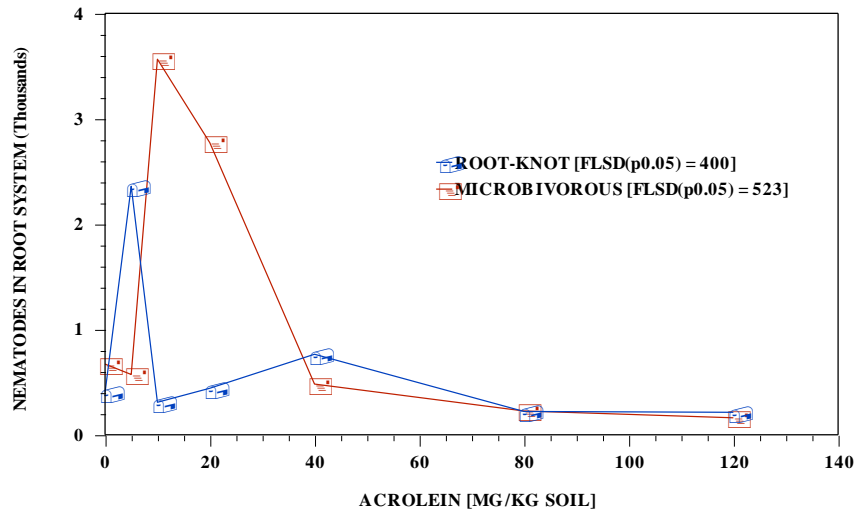


Figure 5C: Root-knot and microbivorous nematodes from tomato roots (Nematode Experiment 5)

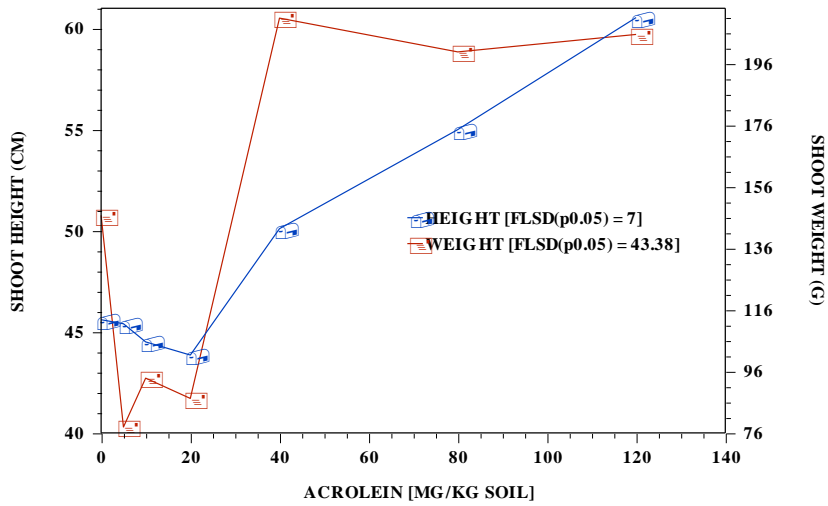
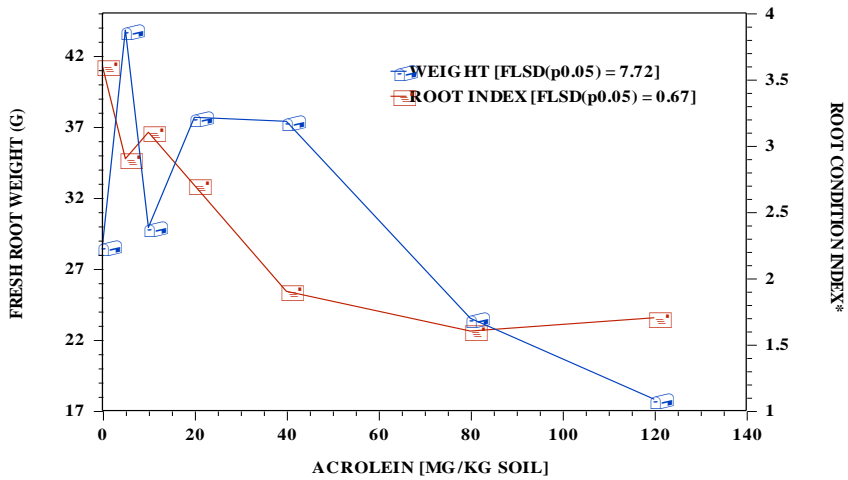
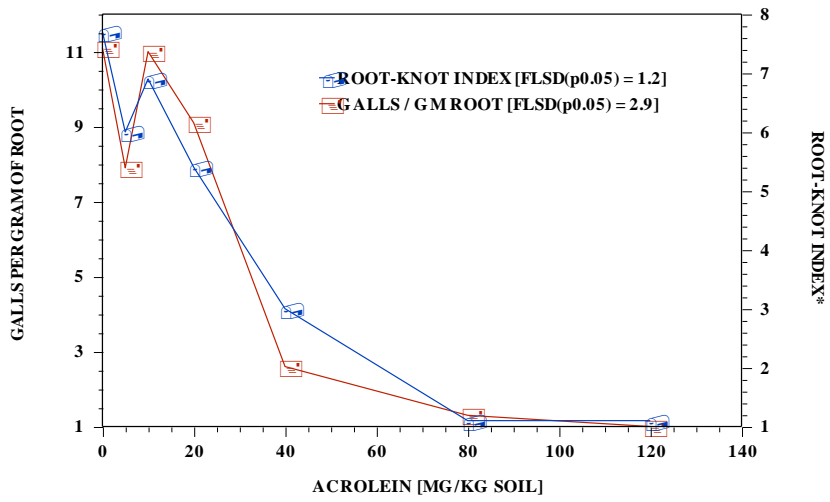


Figure 5D: Tomato shoots heights and weights (Nematode Experiment 5).



*ROOT CONDITION INDEX SCALE: 1= BEST TO 5= WORST

Figure 5E: Tomato root condition rating and weight of fresh shoots (Nematode Experiment 5).



*ROOT-KNOT INDEX SCALE: 0= NO GALLS TO 10 MAXIMUM GALLING

Figure 5F: Root-knot index rating and number of galls per gram of tomato root (Nematode Experiment 5).

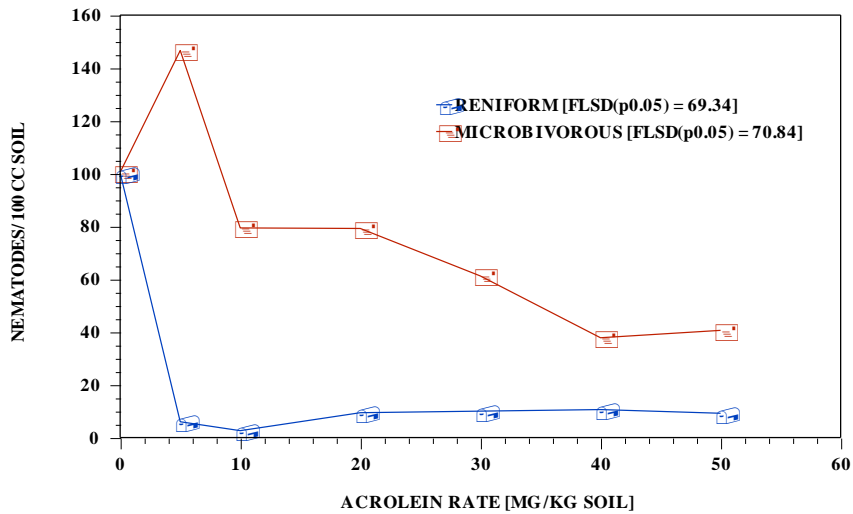


Figure 6A: Reniform and microbivorous nematodes from pre-plant soil sample (Nematode Experiment 6).

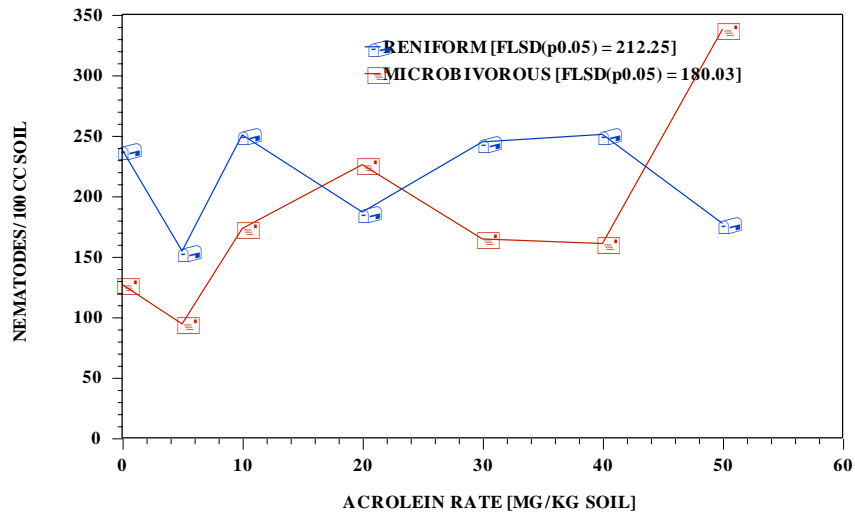


Figure 6B: Reniform and microbivorous nematodes from final soil sample (Nematode Experiment 6).

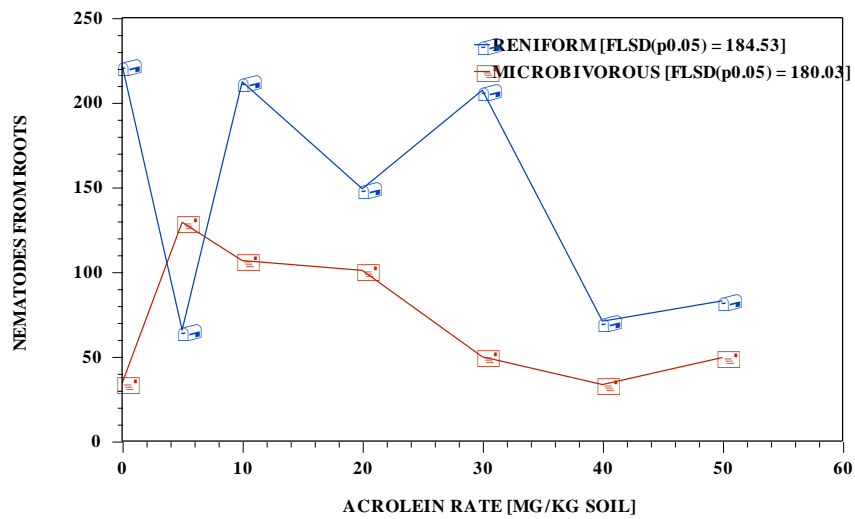
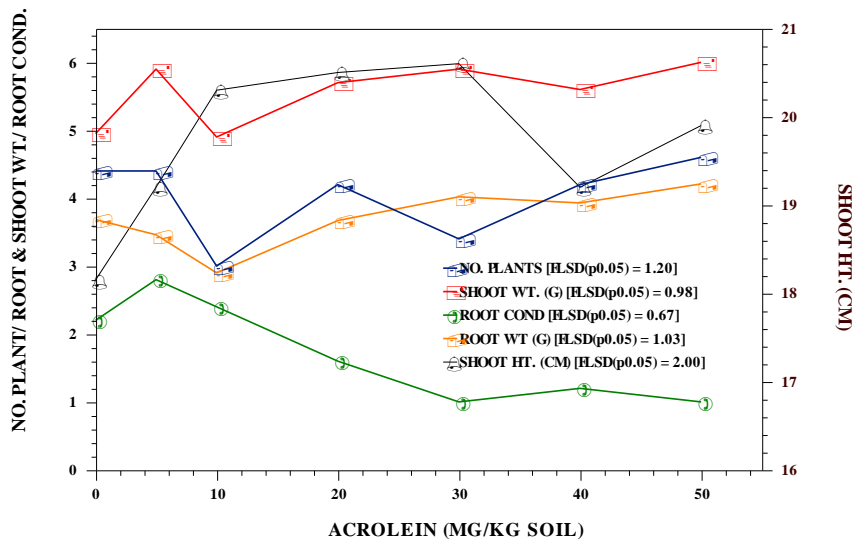


Figure 6C: Reniform and microbivorous nematodes from root sample (Nematode Experiment 6)



ROOT CONDITION SCALE: 1=BEST, 5=WORST

Figure 6D: Cotton plant growth assessments made at the end of the test (Nematode Experiment 6).

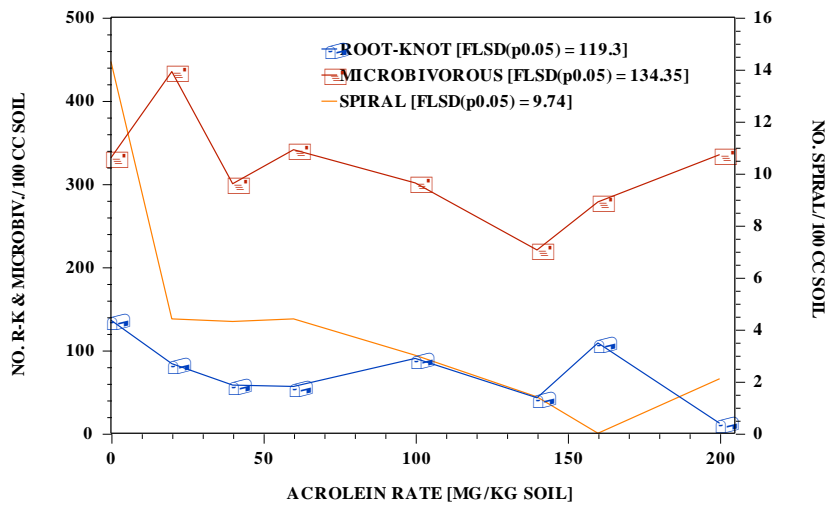


Figure 7A: Root-knot, spiral, and microbivorous nematodes from final soil sample (Nematode Experiment 7).

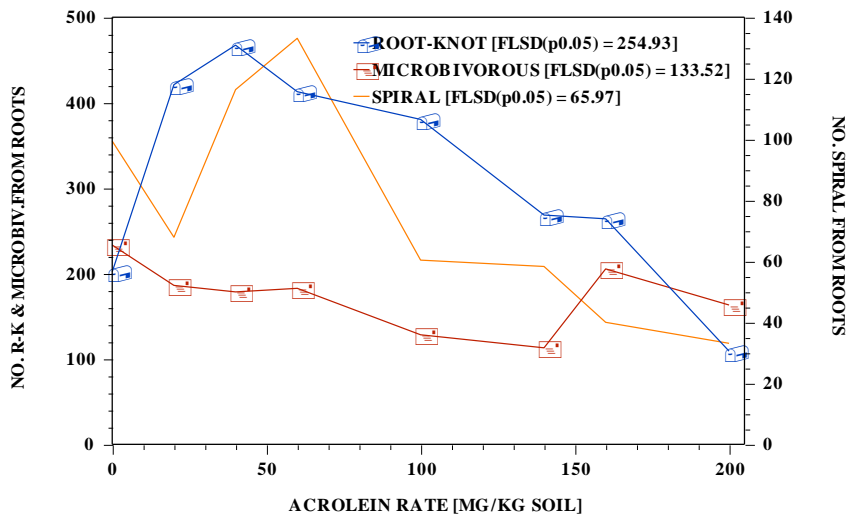


Figure 7B: Root-knot, spiral, and microbivorous nematodes from final root sample (Nematode Experiment 7).

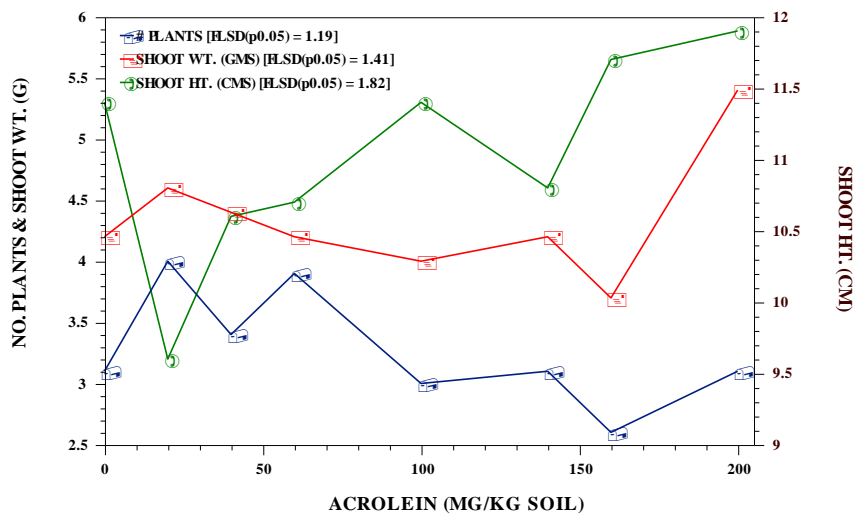


Figure 7C: Number of cucumber plants, weight of shoots in grams, height of shoots in centimeters (Nematode Experiment 7).

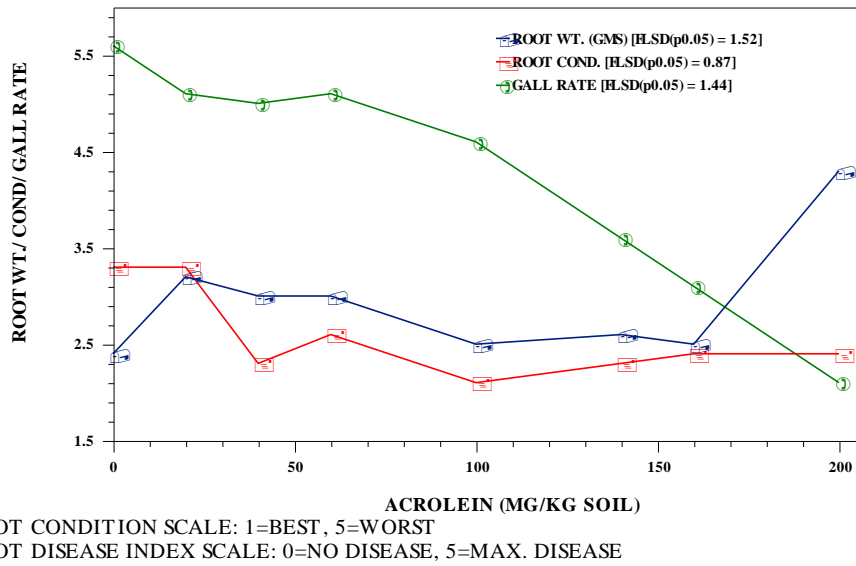


Figure 7D: Weight of roots in grams, root condition rating, and gall rating (Nematode Experiment 7).

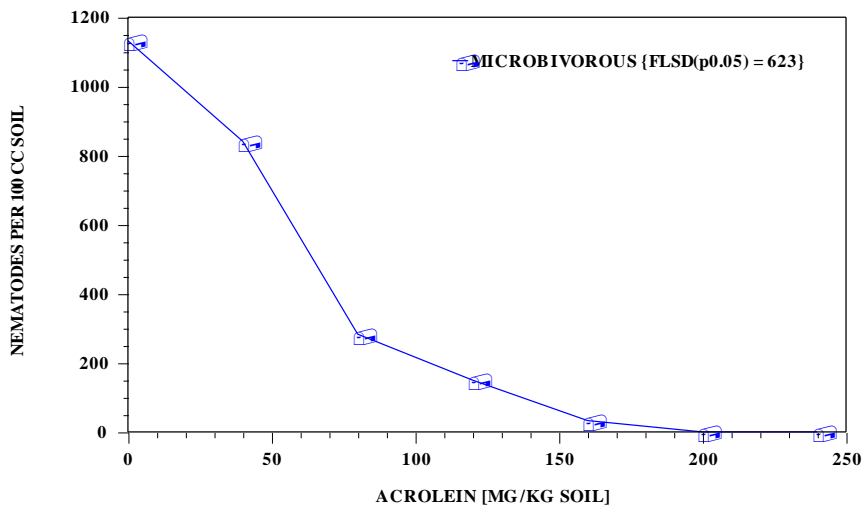


Figure 8A: Microbivorous nematodes from pre-planting soil sample (Nematode Experiment 8).

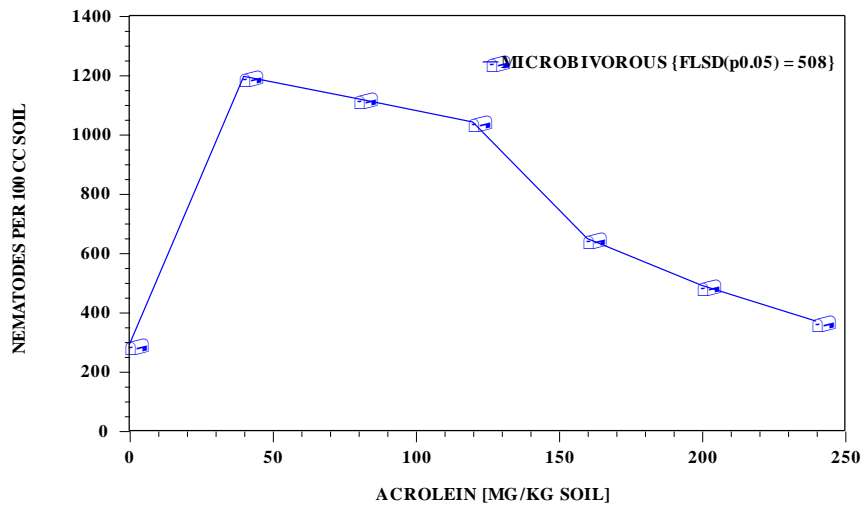


Figure 8B: Microbivorous nematodes from final soil sample (Nematode Experiment 8).

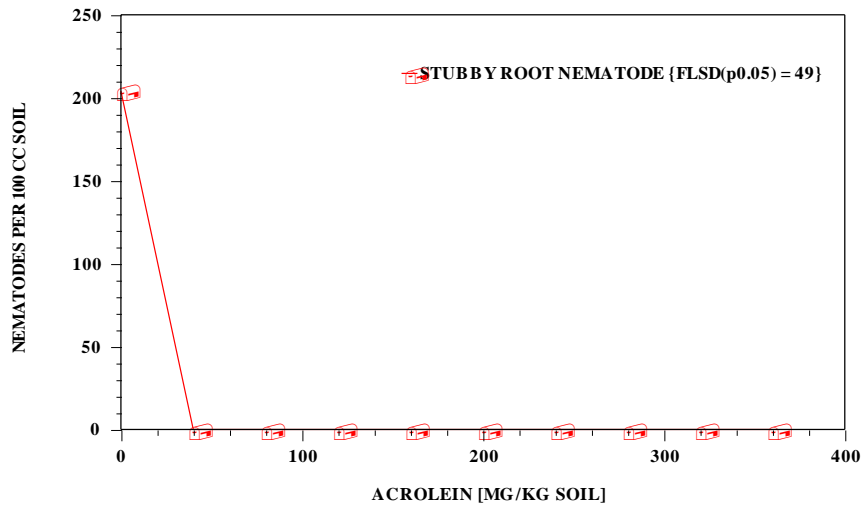


Figure 8C: Stubby root nematodes from final soil sample (Nematode Experiment 8).

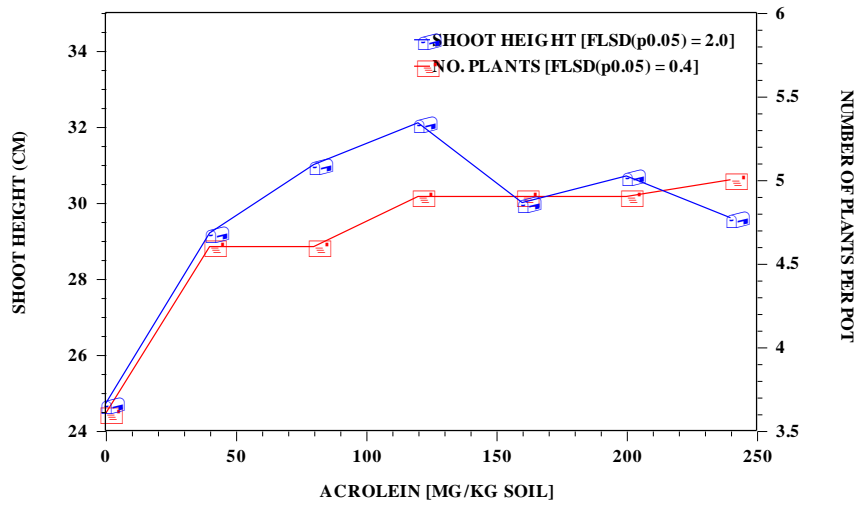


Figure 8D: Number of soybean plants and shoot height from final sampling (Nematode Experiment 8).

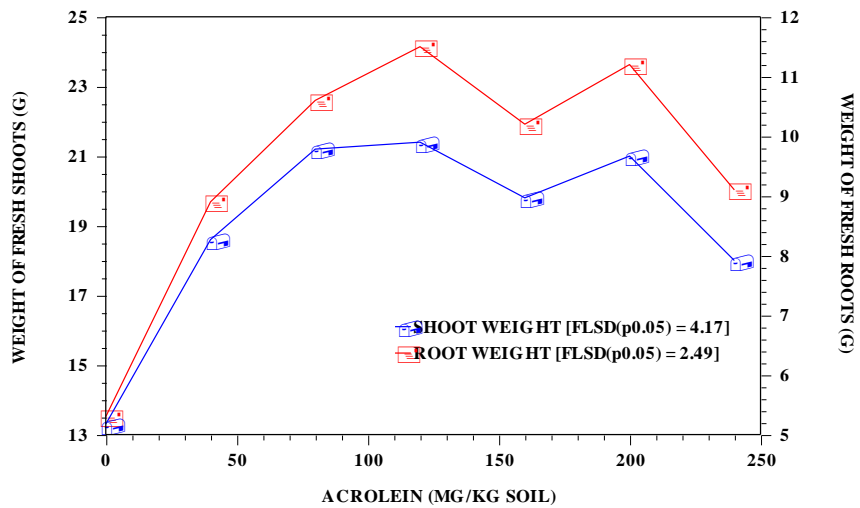
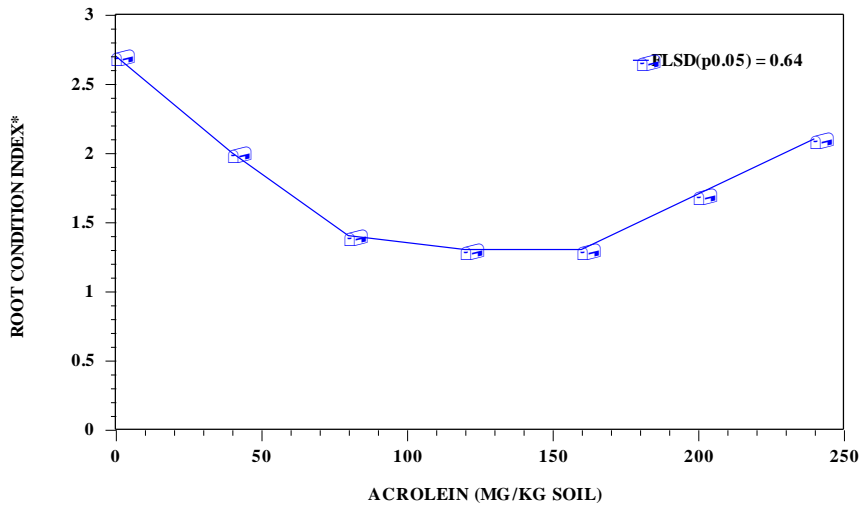


Figure 8E: Fresh soybean shoot and root weight from final sampling (Nematode Experiment 8).



*ROOT CONDITION INDEX SCALE: 1 = BEST APPEARANCE TO 5 = WORST LOOKING

Figure 8F: Soybean root condition rating from final sampling (Nematode Experiment 8).

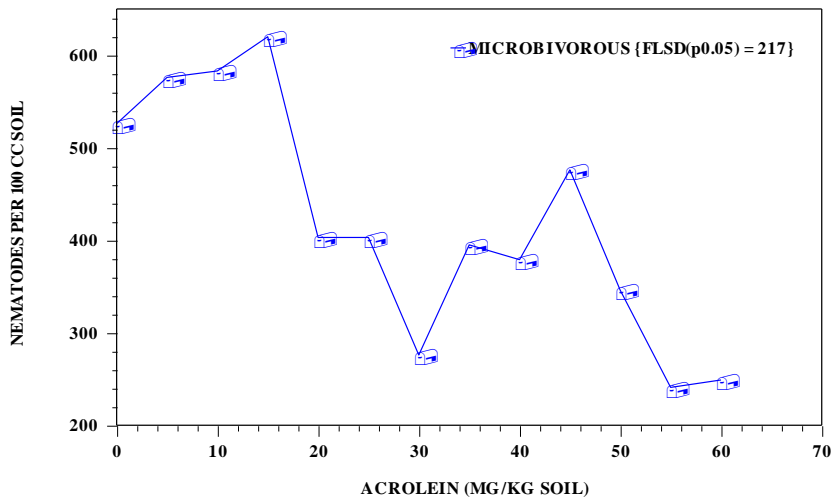


Figure 9A: Microbivorous nematodes from final soil sampling (Nematode Experiment 9).

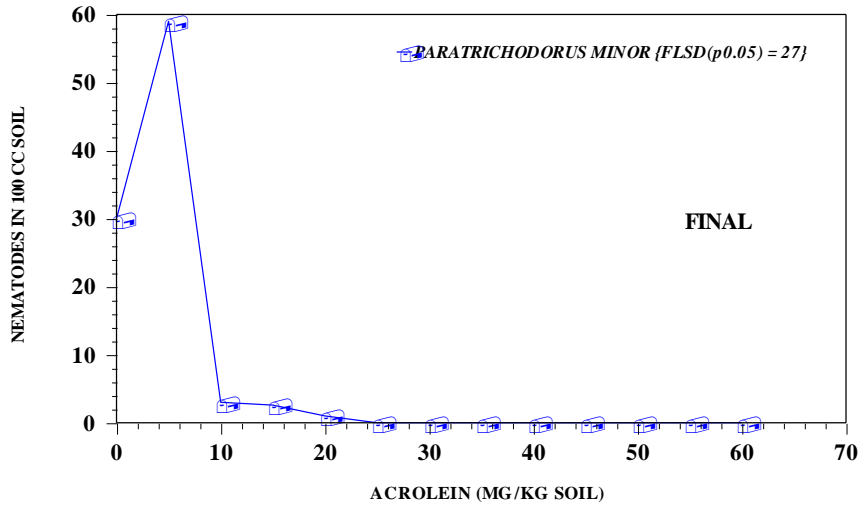


Figure 9B: Stubby root nematode in final soil sample (Nematode Experiment 9).

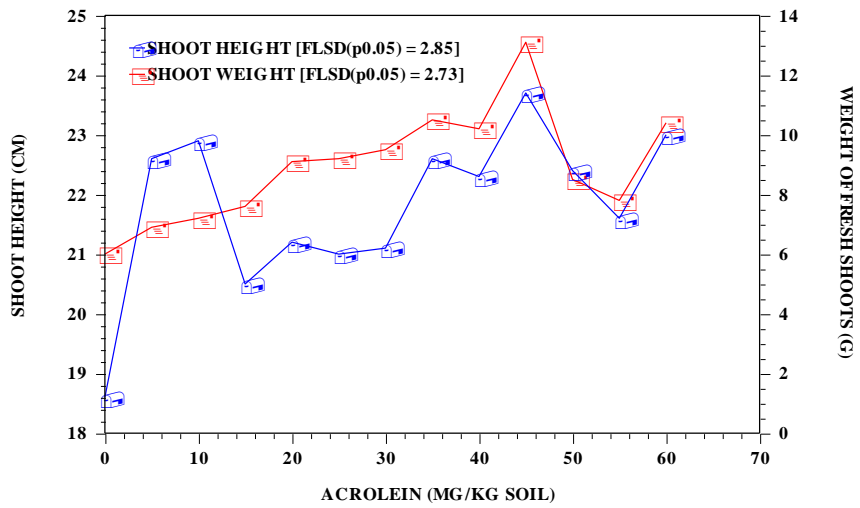


Figure 9C: Fresh soybean shoot height and weight from final sampling (Nematode Experiment 9).

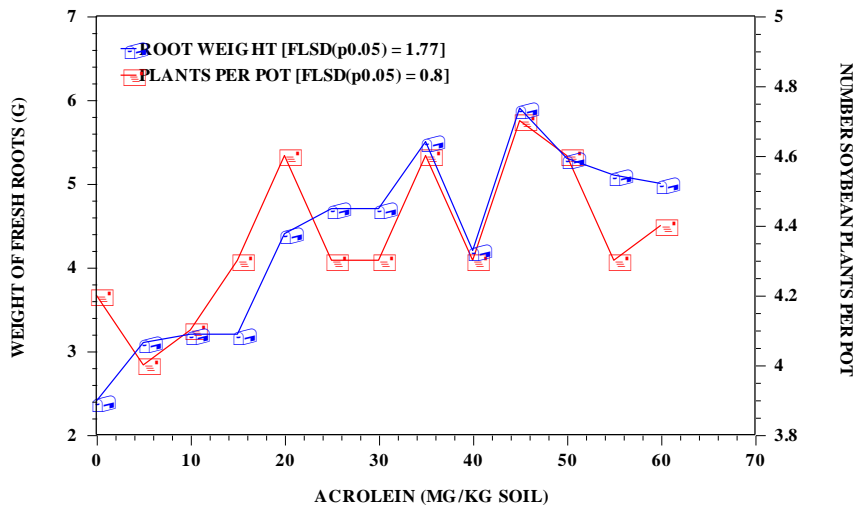
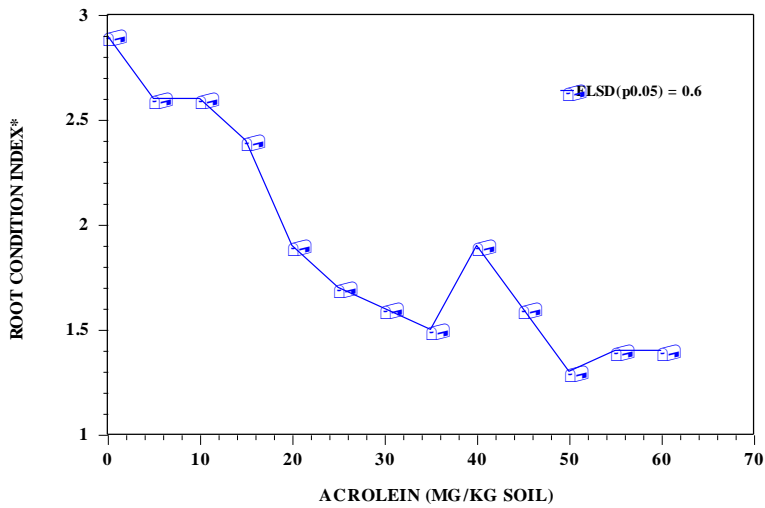


Figure 9D: Number of soybean plants per pot and weight of fresh roots (Nematode Experiment 9).



ROOT CONDITION INDEX SCALE: 1 = BEST APPEARANCE TO 5 WORST LOOKING

Figure 9E: Soybean root condition rating from final sampling (Nematode Experiment 9).

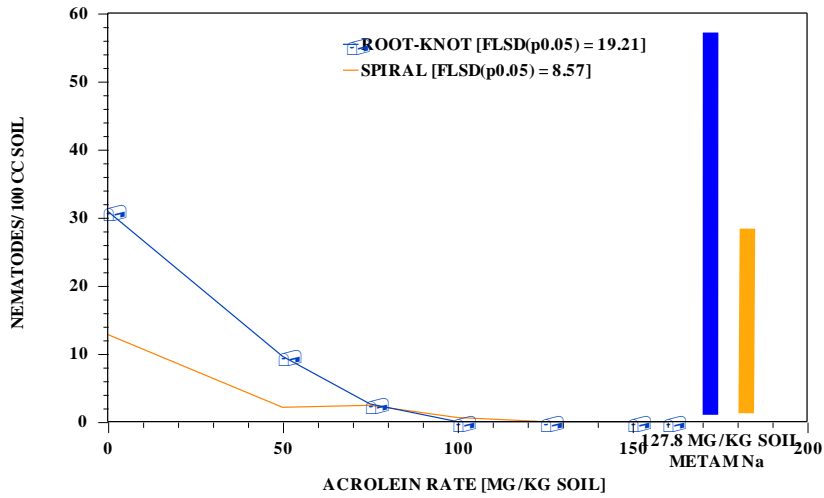


Figure 10A: Number of parasitic nematodes extracted from the soil 119 days after treatment (Nematode Experiment 10).

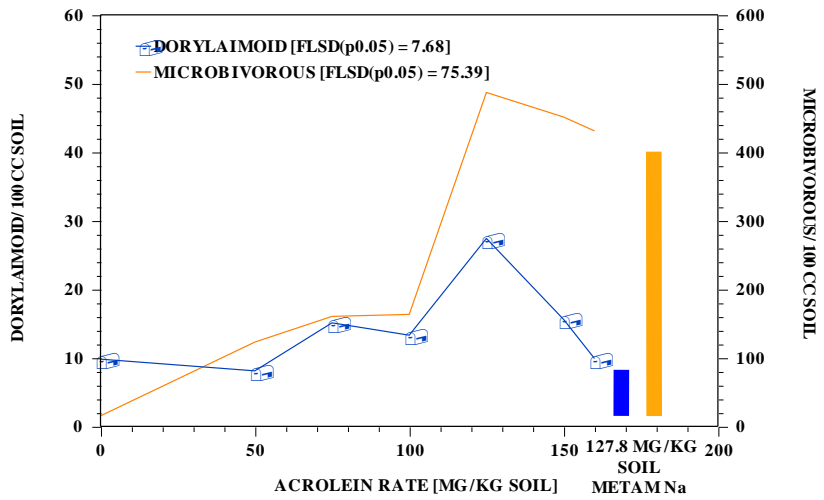


Figure 10B: Number of non-pathogenic nematodes extracted from the soil 119 days after treatment (Nematode Experiment 10).

III. HERBICIDAL PROPERTIES OF ACROLEIN

A. ABSTRACT

The herbicidal properties of acrolein have been known for quite some time. The compound is currently labeled as an aquatic herbicide for use in irrigation canals; however, little has been done to develop strategies and methods to use it in agriculture as an alternative to methyl-bromide. Primary areas of focus in preliminary testing were herbicidal effects on a variety of difficult agronomic weeds. Greenhouse and microplot studies provided efficacy data related to dosimetry and method of application required for field use.

Herbicide efficacy: In greenhouse experiments to determine pre-emergent herbicidal efficacy, morningglory (*Ipomoea lacunose* L./*I. hederacea* Jacq.), sicklepod (*Senna obtusifolia* (L.) H. S. Irwin and Barneby), jimsonweed (*Datura stramonium* L.), large crabgrass (*Digitaria sanguinalis* (L.) Scop.), and yellow foxtail (*Setaria glauca* L. P. Beauv.) were generally controlled with acrolein rates ≤ 100 mg acrolein /kg soil by drench application, while yellow nutsedge (*Cyperus esculentus* L.) required rates ≥ 250 mg for complete control. Because 150% more acrolein was required to control these species, combinations of acrolein with yellow nutsedge-specific herbicides were explored in attempts to reduce rates. Combinations of acrolein with halosulfuron, s-metolachlor, EPTC, butyric acid, and propionic acid were successful for reducing rates; however, it

was also found that when tank-mixed, metam sodium and acrolein were antagonistic. Acrolein was more effective at low rates when applied post-emergence than when used for pre-emergent herbicidal control.

The properties of acrolein make it possible to be used as a fumigant, although rates required for fumigation are higher than those for drench application. In greenhouse fumigation trials, most weeds were controlled with ≥ 400 mg acrolein/kg soil, however, yellow nutsedge required ≥ 600 mg soil for complete control.

Microplot studies showed that drench application of acrolein at rates ≥ 200 mg/kg soil provided good control of weeds 3 weeks after treatment and that treatments ≥ 250 mg were statistically similar to those of metam sodium at 127.8 m/kg soil. Microplot studies also showed that acrolein treatments without plastic cover were herbicidal; however, treatments with cover were significantly more effective.

B. INTRODUCTION

Acrolein (2-propenal; acrylaldehyde), discovered by Redtenbacher in 1843, is one of the oldest compounds synthesized by man (Sanders et. al., 1958). It is found readily in nature as a result of the burning of: fats, fossil fuels, cigarettes, or organic matter; it is also produced by *Bacillus amaracrylus* Voisenet from glycerol (Merck, 1989). Acrolein is a clear-yellow liquid, a lachrymatory fumigant with a vapor pressure within the range adequate for fumigation; it is moderately soluble in water. The herbicidal properties of acrolein have been known for over half a century and it is currently sold as Magnacide-H®, labeled for the control of aquatic weeds and algae. The herbicidal activity of acrolein is reported to be general cell toxicity by reacting with various vital proteins

(Baker Petrolite, 2001). Published work done by McKenry *et. al.* in 1995, as well as U.S. patents No. 3028304 (Kreutzer, 1962), No. 3567776 (Krenzer, 1971), No. 3,052,598 (Racusen and Legator, 1962), No. 5,500,220 (Roe *et al.*, 1996), No. 5866614 (Bockowski and Davis, 1999), No. 6294584 (Bockowski and Davis, 2001), and No. 7255049 (Allan and Schiller, 2007) have alluded to the potential of acrolein for use in agriculture to combat weed pests; however, there is no precise information on the rates and methods of applications needed to control specific weed pests. This study was conducted to determine the specific rates and application methods needed to control several economically important weed pests, including: yellow nutsedge, morningglory, and large crabgrass.

The study consisted of greenhouse and microplots experiments. The greenhouse experiments addressed: (a) herbicidal dosimetry of acrolein applied directly to soil, as a drench and by fumigation, (b) the herbicidal performance of acrolein to control weeds in the presence of heavy weed and pest infestations, and (c) herbicidal efficacy of acrolein when used in combination with organic soil amendments, broad-spectrum pesticides, and herbicides. The microplot experiments investigated; (a) the performance of acrolein compared to metam sodium for control of weeds, (b) herbicidal efficacy of acrolein with and without plastic cover, and (c) herbicidal efficacy of acrolein applied in combination with metam sodium.

C. EXPERIMENTS

Weed Experiment 1: A test was initiated to determine the relationship between acrolein doses applied directly to the soil and herbicidal activity.

Materials and Methods:

The soil used in this experiment (pH 5.6, sandy soil, CEC < 4.6 cmol_ckg⁻¹) was collected from a cotton field at the E.V. Smith Plant Breeding Unit and is naturally infested with large crabgrass and pigweed (*Amaranthus* spp.) seeds. The soil was apportioned into 2 L capacity plastic bags and five yellow nutsedge tubercles were added. Bags were shaken until the contents were evenly blended and then the mixture was transferred into cylindrical plastic pots (10 cm diameter PVC; 1 mm mesh screen bottom; 110 mm diameter student grade Whatman filter paper placed on screen). A 2.5% acrolein solution was prepared by slowly delivering acrolein (90%, Aldrich™) into rapidly vortexing demineralized water. Acrolein at rates: 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, and 300 mg/kg soil was delivered as a 2.5% solution directly into the 2 L bags containing the field soil. The pots were covered with 1 mil low density polyethylene (LDPE) bags secured with rubber bands. Pots were arranged in a randomized complete block design on a greenhouse bench with six replications per treatment. Two non-treated controls were included in the experiment. Bags were removed 8 days after treatment. Weeds were identified and counted at 9, 21, and 25 days after treatment.

Data Analyses:

Data were analyzed using SAS software; Fisher's least significant differences (P=0.05) were calculated when F values were significant. Unless otherwise noted, statements made in the results of this experiment and all others in this chapter are for significant differences at P=0.05 level of probability.

Results:

Pots of soil treated with acrolein at rates ≥ 125 mg/kg soil had no large crabgrass at 9 and 21 days after treatment (Fig. 11A). There were no pigweeds in pots of soil treated with ≥ 75 mg acrolein 9 days after treatment; pots treated with 100, 175, 200, 225, 250, and 275 mg had no pigweeds 21 days after treatment (Fig. 11B). Populations of large crabgrass and pigweeds were low in this experiment. Acrolein treatments had little significant effect on yellow nutsedge only providing complete control at 275 and 300 mg/kg soil at 9 days after treatment (Fig. 11C). Lower rates of acrolein tended to stimulate yellow nutsedge. The number of total weeds consisted primarily of yellow nutsedge, so trends and significance was similar to that of the yellow nutsedge data alone (Figs. 11C and 11D).

Conclusions:

Acrolein provided control of large crabgrass and pigweed at rates acceptable for field use. Acrolein was inadequate for control of yellow nutsedge at the rates used in this experiment.

Weed Experiment 2: A second experiment was conducted similarly to Weed Experiment 1 to confirm findings on the herbicidal activities of acrolein applied directly to the soil. Weed counts were taken over 1 month after treatment to monitor the herbicidal effects of acrolein over a longer duration than in the previous experiment.

Materials and Methods:

The experiment was as described in Weed Experiment 1. Pots were uncovered 7 days after treatment and weed counts were taken at 15, 25, 32, and 42 days after treatment.

Results:

Large crabgrass was stimulated by acrolein rates in the 25 - 200 mg range throughout the experiment (Fig. 12A). Pigweed was reduced in pots treated with 25 mg acrolein and stimulated in pots treated with 75 mg acrolein (Fig. 12B). Pigweed was controlled 42 days after treatment in pots treated ≥ 175 mg/kg (Fig. 12B). Pots treated with 75 mg acrolein increased yellow nutsedge ($P=0.10$) (Fig. 12C). Acrolein rates above 75 mg decreased the number of yellow nutsedge (Fig. 12C). Total weed numbers consisted primarily of pigweed (Figs. 12B and 12D).

Conclusions:

Acrolein stimulated large crabgrass at low rates. Low rates of acrolein also stimulated pigweed while high rates provide excellent control over time. Low rates of acrolein tended to stimulate nutsedge ($P=0.10$) while the high rates used in this experiment caused a reduction but did not result in complete control.

Weed Experiment 3: To determine the direct application rates required to completely control yellow nutsedge, a weed test similar to Weed Experiment 1 was conducted, except with higher rates of acrolein.

Materials and Methods:

The experiment was conducted as described for Weed Experiment 1 but the acrolein treatments were with 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, and 600 mg/kg soil. Pots were uncovered 7 days after treatment and weed counts were taken at 12, 20, 28, and 35 days after treatment.

Results:

No large crabgrass was observed in the first count; however, in the second weed count there was stimulation of large crabgrass in pots treated with 100 and 150 mg acrolein (Fig. 13A). Acrolein at rates ≥ 300 mg/kg soil suppressed large crabgrass populations (Fig. 13A). The number of pigweed decreased as rates of acrolein were increased (Fig. 13B). No pigweed plants were found in pots treated with ≥ 300 mg acrolein 35 days after treatment (Fig. 13B). With the exception of the 100 mg rate, as rates of acrolein increased the number of yellow nutsedge plants tended to decrease (Fig. 13C). There was no nutsedge 12 days after treatment in pots treated with ≥ 300 mg acrolein and none at 35 days in pots treated with ≥ 450 mg acrolein (Fig. 13C). While all rates of acrolein reduced the total number of weeds in the first count 12 days after treatment, by the final count at 35 days, pots treated with 100 mg acrolein had more weeds than the controls (Fig. 13D). No weeds were found in pots treated with ≥ 300 mg acrolein 12 days after treatment; however, all acrolein treatments had some weeds 35 days after treatment (Fig. 13D).

Conclusions:

Using much higher rates than in the previous two experiments showed that there is little improvement in large crabgrass control with rates over 300 mg/kg soil. Pigweeds

are completely controlled with 200 - 300 mg acrolein. Rates of 450 mg of acrolein are required to provide complete control of yellow nutsedge for 1 month after treatment.

Weed Experiment 4: The herbicidal activity of drench applications of acrolein to soil was studied with one pre-plant experiment and two post-emergent experiments. This experiment studied pre-plant drench applications of acrolein on a variety of weed species.

Materials and Methods:

One kg aliquots of Tallassee field soil were apportioned into 2 L plastic bags. Into plastic cups, 10 nutsedge tubercles (a perennial sedge), ~ 112 yellow foxtail seed (an annual grass), ~ 27 morningglory seed mix (an annual broad-leaf species), ~ 40 sicklepod seeds, two cocklebur seeds (*Xanthium strumarium* L.) (an annual broad-leaf species), and ~ 66 jimsonweed (an annual broad-leaf species) seeds were mixed to supplement the natural weed populations of the soil. One cup of seeds, or “weed pack” was added to each of the 1 kg bags of soil. The bags were shaken until the seeds were evenly mixed and the contents were deposited into the pots. A 1% acrolein solution was prepared by slowly adding 5 ml of acrolein (Baker-Petrolite™) into 495 ml of vortexing demineralized water. Acrolein was applied at 75, 150, and 300 mg/kg soil, each in final drench volumes of 30, 50, 75, and 100 ml using water. All pots were covered and arranged on a greenhouse bench in a randomized complete block design with seven replications per treatment. Two water-treated controls were included. Pots were uncovered 6 days after treatment and weed counts were taken at 7, 11, 18, 27, 32, and 49 days after treatment.

Results:

There were no differences among acrolein treatments for the 75 and 150 mg rates throughout the test so only data from 300 mg treatments will be presented. Pots treated with acrolein had fewer large crabgrass plants per pot than the controls (Fig. 14A). While there were some significant differences in the number of large crabgrass plants per pot, there was no clear trend throughout the test related to the volume of the drenches (Fig. 14A). Morningglory was completely controlled with this rate of acrolein, regardless of drenching volume (Fig. 14B). While all pots treated with acrolein had fewer sicklepod plants per pot, the numbers were also not dramatically affected by the final volume of drench used (Fig. 14C). At 18 days after treatment, pots drenched with 300 mg acrolein in a final volume of 50 ml had fewer nutsedge than pots drenched with 300 mg acrolein in the other final volumes; there were no other consistent patterns observed for the other final volumes (Fig. 14D). All acrolein-treated pots had fewer total weeds than the control; however, the only differences among drenching volumes was noted in the last two weed counts where the 30 ml drench had more total weeds than the other drench volumes.

Conclusions:

Drench applications of acrolein in aqueous solution proved to be effective at controlling weeds. Differences were observed only with the final drench volume of the 300 mg rate. With this rate, acrolein applied in 50 ml final volume provided the optimum control of weeds.

Weed Experiment 5: Two experiments were designed to explore the efficacy of drench applications of acrolein for post-emergence weed control. This experiment was conducted with mature yellow nutsedge plants to determine efficacy and dosimetry.

Materials and Methods:

Pots with Tallassee field soil and 10 - 15 nutsedge plants were used in the experiment. Acrolein was drench applied at rates: 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 mg/kg soil. Drenches were made using appropriate amounts of 10% stock acrolein solution delivered into 100 ml plastic cups, bringing final volume to 100 ml with tap water, and pouring the solution evenly over the soil surface in the pots. The pots were covered with 1 mil low density polyethylene (LDPE) bags secured with rubber bands. Pots were arranged in a randomized complete block design on a greenhouse bench with six replications per treatment. Two non-treated controls were included in the experiment. Bags were removed 8 days after treatment. Yellow nutsedge survival ratings were taken 12 days after treatment (rating scale 1 - 5; 5= all plants dead, 1= all plants green). Weed counts were taken 30 and 48 days after treatment. Five 'Hutcheson' soybean (*Glycine max* 'Hutcheson') seeds were planted into each pot (with weeds) 30 days after treatment. Soybean emergence counts were taken 18 days after planting.

Results:

All pots treated with acrolein had fewer nutsedge survive than the control (Fig. 1A) and those with acrolein ≥ 200 mg had no nutsedge plants surviving 12 days after treatment. Pots treated with 100 mg of acrolein had fewer nutsedge plants than the controls 30 days after treatment and pots treated with acrolein ≥ 200 mg soil had fewer

nutsedge than those treated with 100 mg (Fig. 15A). Similar results were observed 48 days after treatment (Fig. 15A). There were no differences in soybean emergence among any of the acrolein treatments or controls (Fig. 15B).

Conclusions:

Drench applications to yellow nutsedge plants resulted in excellent control at \geq 200 mg acrolein. No phytotoxicity was noted when soybeans were planted after treatment, indicating the potential for aqueous solutions of acrolein for post-emergence control of difficult weeds.

Weed Experiment 6: A second experiment was conducted to assess post-emergence herbicidal activities of drench-applied acrolein alone and in combination with H-cyanamide.

Materials and Methods:

Experimental units consisted of 10 cm plastic pots containing 1 kg Tallassee, AL field soil with 9 - 10 yellow nutsedge plants in each one. A 2% acrolein solution was prepared by using 22 ml 90% acrolein (Aldrich™) in a final volume of 1000 ml with tap water. A 5% cyanamide solution was prepared using 100 ml of 50% cyanamide (Aldrich™) in a 1000 ml final volume with tap water. Appropriate amounts of the stock solutions were delivered into plastic cups and the final volume was brought up to 100 ml with tap water. Treatments were drench applied in 100 ml/pot and 100 ml water was applied to the controls. Acrolein was applied alone at 50, 100, 150, and 200 mg/kg soil. Acrolein was also applied at the same rates with the addition of 250 mg cyanamide/kg soil. A control with cyanamide alone at 250 mg/kg soil was included. Immediately

following drenching, pots were covered with 1 mil thick plastic bags and then sealed with rubber bands. Seven replications were used per treatment and two controls were included. Pots were arranged on a greenhouse bench in a randomized complete block design. Pots were uncovered 4 days after treatment and weed counts were taken at 7, 14, 22, and 29 days after treatment.

Results:

All pots treated with acrolein alone had an average of less than one yellow nutsedge plant per pot (Fig. 16A). All pots treated with the combination of 50 mg acrolein and 500 mg cyanamide had more nutsedge plants per pot than pots treated with 50 mg acrolein alone (Fig. 16A). The number of nutsedge plants in the combination treatments began to rise at the final weed count; however, the number of nutsedge in pots treated with acrolein alone remained relatively unchanged throughout the experiment.

Conclusions:

Drench applications of acrolein alone were better for controlling germinated yellow nutsedge plants than combinations with cyanamide at 250 mg/kg soil. Acrolein provided excellent control of yellow nutsedge using this method of treatment.

Weed Experiment 7: With a vapor pressure of 325.7mm Hg at 30°C, acrolein has potential to be used as a fumigant like methyl bromide. Three fumigations experiments were developed to explore this possibility. These experiments were conducted using specialized greenhouse fumigation chambers.

Materials and Methods:

Acrolein in a 2.5% aqueous solution was delivered into the bottoms of greenhouse fumigation chambers (Fig. 17A, Labeled C) at rates: 0, 100, 200, 300, 400, 500, 600, 800, and 1000 mg/kg soil. Each chamber consisted of a glass jar (approx. 1 L) with a 1 kg-size PVC pot sealed to the top (Fig. 17A, Labeled B and A). Immediately after the delivery of each treatment, a Whatman filter paper was placed at the bottom of each pot (Fig. 17A, Labeled D) and 1 kg of Tallassee field soil with five nutsedge tubercles was pored into each pot. Pots were then covered with a 1 mil thick plastic bag, sealed using a rubber band, and were arranged on a greenhouse bench in a randomized complete block design. Six replications were used per treatment and two non-treated controls were included. Bags were removed 10 days after treatment. Weed counts were taken 10, 17, 25, 40, 47, and 57 days after treatment. The presence of *Trichoderma* spp. on the soil surface and/or nutsedge tubercles was recorded 20 days after treatment.

Results:

At each weed count, all pots treated with acrolein had virtually no large crabgrass nor pigweed (Fig. 17B and 17C). Yellow nutsedge plants were fewer in all pots treated with acrolein as compared to the controls; pots treated with ≥ 200 mg/kg soil had no nutsedge 10 days after treatment (Fig. 17D). No nutsedge plants were noted in any pots treated with ≥ 300 mg acrolein 52 days after treatment and the numbers of nutsedge in pots treated with ≥ 200 mg acrolein were statistically similar to those pots with no nutsedge (Fig. 17D). At 25 days after treatment, no weeds were noted in any pots treated with ≥ 300 mg acrolein and after 52 days no weeds were noted in any pots treated with ≥ 400 mg/kg soil (Fig. 17E). The number of soybean seedlings was improved by

treatments in the range of 200 - 500 mg acrolein at 7, 14, and 24 days after planting (Fig. 17F). *Trichoderma* colonies were observed on the soil surface of each acrolein rate used, and there were colonies in every replication of pots treated with 200 - 400 mg acrolein (Fig. 17G). Nutsedge tubercles were infested with *Trichoderma* spp. in pots treated with acrolein at rates: 100, 400, 500, 600, and 1000 mg/kg soil (Fig. 17G).

Conclusions:

Acrolein can be used to fumigate soils to control weeds. Rates that provided excellent weed control also increased soybean germination. Effective acrolein rates were similar to the effective rates of direct application to soil. The stimulation of *Trichoderma* spp. is an indication that acrolein may improve the soil microbiology by selecting for non-pathogenic microorganisms.

Weed Experiment 8: An experiment was setup to measure the herbicidal effects of fumigation with acrolein in varying concentrations and volumes of fumigant solutions. Acrolein has an affinity for water suggesting potential of aqueous solutions to prolong the herbicidal activity of acrolein.

Materials and Methods:

Specialized greenhouse fumigation chambers were developed for this series of acrolein experiments using the standard 10 cm diameter PVC pipe and screen (Figs. 18A and 8B). The upper portion of the chamber (Fig. 18A, Labeled A) is the same height (18 cm) as the standard greenhouse pots so that it may contain 1 kg soil. The lower portion of the chamber (Fig. 18A, Labeled B) is 7.5 cm tall so that a 7.2 cm tall ring, or “basal fumigation ring” (Fig. 18A, Labeled C), with bag (Fig. 18A, Labeled D) and fumigant

can be placed inside. The fumigation ring was cut vertically (Fig. 18A, Labeled C) so that it would fit tightly in the bottom to prevent fumigant escape (Fig. 18B). A 20.5 X 20.5 cm zip-lock bag (Fig. 18A, Labeled D) is placed in the 7.2 cm tall ring to contain the fumigant solution. One kg aliquots of Tallasse weed with previously described “weed pack” was placed into the upper portion of the fumigation chambers (Fig. 18A, Labeled A). Pots were watered to field capacity, the chambers were covered with 2 mil plastic bags, and they were then sealed with a rubber band. A 2% acrolein solution was made by slowly adding 20 ml of 99% acrolein to rapidly vortexing demineralized water and bringing up to a final volume of 1000 ml. Acrolein was applied at three rates (80, 160, and 240 mg/kg soil) each in four different volumes of water (100, 200, 300, and 400 ml). Appropriate amounts of water were added to the zip-lock bag in the fumigation rings followed by the appropriate amount of acrolein. After each basal fumigation ring received the proper amount of water and acrolein, the rest of the fumigation chamber with weed soil was immediately slipped over the top, thus sealing the chamber (Fig. 18B). Pots were arranged in a randomized complete block design on a greenhouse bench using seven replications. Two non-treated controls were included in this experiment. Pots were uncovered 3 days after treatment and a weed count was taken 11 days after treatment.

Results:

Few and small differences were noted among treatments for any of parameters measured (Fig. 18C and Table 2).

Conclusions:

Fumigation of soil at field capacity with acrolein was not effective for weed control. The data suggests that soil moisture level may be critical for the movement of the chemical through soil.

Weed Experiment 9: A second fumigation experiment was conducted similar to Weed Experiment 8 but with soil moisture content at ½ field capacity.

Materials and Methods:

The experiment was conducted as for Weed Experiment 8, but with soil at ½ field capacity. Pots were uncovered 3 days after treatment and weed counts were taken 6, 8, 10, and 16 days after treatment.

Results:

The number of large crabgrass plants was reduced by all acrolein treatments at each weed count except for the 80 mg rate of acrolein (Fig. 19A and 19B). All pots treated with acrolein had fewer morningglory plants than the controls throughout the trial (Fig. 19A and 19C). All acrolein treatments, except the 80 mg rate in 200 ml water, reduced the number of pigweeds (Fig. 19A and 19D), and all acrolein treatments reduced the number of sicklepod (Fig. 19A and 19E). Pots treated with 80 mg acrolein in 100, 300, and 400 ml water had more of yellow nutsedge plants per pot as compared to the controls in the first count; pots treated with 160 mg acrolein in 300 and 400 ml water also had more nutsedge than the controls (Fig. 19A and 19F). Acrolein treatments reduced the total number of weeds (Fig. 19A and 19G); however, 60 mg acrolein in 100 ml water had significantly fewer weeds than the same rate in 300 and 400 ml water (Fig. 19G). All

pots treated with 240 mg acrolein had significantly fewer weeds than those treated with 80 mg acrolein, regardless of the amount of water used (Fig. 19G).

Conclusions:

High fumigation rates of acrolein resulted in the best herbicidal efficacy. As the amount of water in the solution increased, the efficacy of acrolein decreased. Results indicate that when using acrolein in aqueous solution to fumigate, the amount of water should be reduced to the least possible.

Weed Experiment 10: Two experiments were designed to determine the efficacy of acrolein in soils with multiple pest pressures. As such, these “extreme tests” served to complement data obtained from previous single pest experiments. In this test, the herbicidal efficacy of drench-applied acrolein was explored using soil with multiple fungal, nematode, and weed pests.

Materials and Methods:

The soil and soil preparation was as described in Nematode Experiment 8. Acrolein was applied at: 40, 80, 120, 160, 200, 240, 280, 320, 360, 400, 440, and 480 mg in a final drench volume of 100 ml per pot. Controls were treated with 100 ml water only. Drench solutions were made using a stock 2% acrolein solution. Pots were covered with plastic bags and sealed with rubber bands immediately following treatment. The plastic bags were removed 1 week after treatments and weed counts were taken at 7, 13, and 20 days after treatment. The weeds were then removed, a soil sample was taken for nematode analysis (salad bowl technique), and each pot was planted with five ‘Hutcheson’ soybean seeds. The soybeans were allowed to grow for 6 weeks when they

were removed from the pots for plant growth assessment and final nematode sampling. Plant growth parameters determined were: number of plants, height of shoots (cm), weight of fresh shoots (g), weight of fresh roots (g), and a root condition rating scale (1 - 5; 1= best, 5= worst).

Results:

Nematode and soybean growth data were presented in Chapter 2. All pots treated with acrolein reduced numbers of large crabgrass plants throughout the test (Fig. 20A). No large crabgrass was detected in any pot treated with ≥ 120 mg acrolein in all three weed counts (Fig. 20A). For all three weed counts, morningglory counts were lowest in pots treated with acrolein (Fig. 20B). There were more sicklepod in the controls than any acrolein treatment in the first weed count (Fig. 20C). With low numbers of sicklepod in the controls, there were no significant differences between the controls and the acrolein-treated pots in the second count (Fig. 20C). By the third count, pots treated with 40, 120, 200, and 400 mg acrolein had statistically similar numbers of sicklepod as the controls (Fig. 20C). In the first count, all pots treated with ≥ 120 mg acrolein reduced numbers of yellow nutsedge plants (Fig. 20D). In the second and third counts, pots treated with ≥ 160 mg acrolein had fewer yellow nutsedge than the controls while those treated with 40 mg acrolein had a greater number of yellow nutsedge plants per pot (Fig. 20D). The total number of weeds was lower in all acrolein-treated pots in each of the three weed counts (Fig. 20E).

Conclusions:

Acrolein provided good control of large crabgrass and morningglory at rates 40 - 100 mg/kg soil. Over twice the amount of acrolein was required to control yellow nutsedge than was required to control large crabgrass and morningglory.

Weed Experiment 11: A second test was setup to determine the herbicidal efficacy of acrolein in multiple pest infestations. In this experiment, lower doses were used to determine, specifically, what rates are required to control the weed species under study.

Materials and Methods:

The test setup was identical to Weed Experiment 10 except the acrolein treatment rates were: 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 mg/kg soil and were prepared from a 0.25% acrolein stock solution. All assessments and analyses remained the same as the previous experiment except that weeds were counted 7, 15, and 21 days after treatment.

Results:

The number of large crabgrass plants declined in response to acrolein in a linear manner (Fig. 21A). A similar response pattern was observed for sicklepod and morningglory, except that the declines were sharper and non-linear; the most acute drop in weed numbers occurring in the range of 5 - 20 mg of acrolein (Figs. 21B and 21C). Acrolein failed to control yellow nutsedge; the 55 mg rate of acrolein may have stimulated the weed (Fig. 21D). The total number of weeds was reduced with increasing doses of acrolein in a linear manner, similar to that observed for large crabgrass (Fig. 21E).

Conclusions:

Morningglory and sicklepod were more sensitive to the chemical than large crabgrass and yellow nutsedge was relatively unaffected by acrolein applications.

Combination Experiments with Naturally Occurring Compounds: In attempts to improve herbicidal efficacy and reduce the rates of acrolein required, a series of tests were setup with acrolein treatments being applied in combination with other compounds. Three experiments were designed to determine the effects of acrolein when applied in combination with naturally occurring compounds.

Weed Experiment 12: This experiment explored combinations of acrolein with glycerin, a compound readily found in nature. Glycerin is easily decomposed in soil and can serve as a carbon source for bacteria, actinomycetes, and fungi in the genera *Trichoderma*, *Gliocladium*, and other fast growing species.

Materials and Methods:

Soil, weed seed, and pot preparations were as for Weed Experiment 10. A 2% stock acrolein solution was prepared and a 20% glycerin solution was prepared by adding 100 ml glycerin to 400 ml demineralized water. Acrolein was applied at 40, 80, 120, 160, 200, 240 mg/kg soil. Combination treatments will be presented in the format mg acrolein: mg glycerin. The combinations were: 40:400, 80:800, 120:2400, 160:3200, 200:4000, and 240:4800. Pots were uncovered 6 days after treatment and weeds were counted 6, 9, 13, and 22 days after treatment. *Trichoderma* spp. ratings were taken 13 days after treatment (0 - 5 rating scale; 0= no *Trichoderma*, 5= maximum *Trichoderma*).

Results:

Few differences were observed except for *Trichoderma* data. *Trichoderma* spp. were noted on the soil surface in several pots of every treatment containing acrolein except the 40:400 and 80:800 combinations (Fig. 22A). Ratings were significantly greater in pots treated with 200 and 240 mg acrolein as well as 160:3200, 200:4000, and 240:4800 combinations (Fig. 22A). The most *Trichoderma* was noted in pots treated with the 200:4000 combinations (Fig. 22A). Few and small differences in herbicidal activity were observed when acrolein was drench-applied with glycerin (Figs. 22B, 22C, 22D, and 22E).

Conclusions:

There were no large improvements in herbicidal efficacy when applying acrolein in combination with glycerin. The addition of glycerin to acrolein treatments did increase the *Trichoderma* colonies suggesting that the inclusion of glycerin may help improve the soil microflora.

Weed Experiment 13: A second experiment was conducted using acrolein with naturally occurring compounds. Propionic acid, a natural product of anaerobic bacterial fermentation, has herbicidal properties, promotes *Trichoderma* spp, and is fungistatic to other fungi when added to soil.

Materials and Methods:

Tallassee, AL field soil was prepared as for Weed Experiment 8 except that 5 nutsedge tubercles were in each supplement of weeds. Soil with supplemental weed seeds as described in Weed Experiment 4, were placed into standard 10 cm PVC pots and

placed in the greenhouse for treatment. A 1% acrolein solution was prepared by slowly adding 5 ml of acrolein (Baker-Petrolite™) into 495 ml of vortexing demineralized water. A 5% propionic acid solution was prepared by adding 25 ml propionic acid (Aldrich™) into 475 ml of vortexing demineralized water. Using these stock solutions, final solutions containing the appropriate amount of each compound were prepared to deliver in a final volume of 100 ml/pot. The treatments were drench applied. Acrolein was applied alone at 20, 40, 60, 80, and 100 mg/kg soil, and the same rates were applied in combination with 250 mg propionic acid. Propionic acid was also applied alone at 250 mg/kg soil. Controls treated with 100 ml water alone were included. All pots were covered after treatment and arranged in a randomized complete block design with seven replications on a greenhouse bench. Pots were uncovered 5 days after treatment and weed counts were taken at 5, 10, and 17 days after treatment.

Results:

The addition of propionic acid to acrolein treatments resulted in increased control of large crabgrass, morningglory, and sicklepod (Figs. 23A, 23B, 23C). Propionic acid alone at 250 mg had no effect on large crabgrass, controlled morningglory throughout the test, and had little effect on sicklepod (Figs. 23A, 23B, 23C). The addition of propionic acid to acrolein treatments improved control of yellow nutsedge at the second count; however there were no significant differences between acrolein treatments alone and those with propionic acid in later counts (Fig. 23D). Yellow nutsedge in the first weed count was stimulated by the 20 and 40 mg rates of acrolein alone (Fig. 23D); propionic acid alone at 250 mg also stimulated nutsedge (Fig. 23D). All acrolein treatments except

the 100 mg rate, resulted in fewer total weeds per pot when applied with propionic acid than when applied alone (Fig. 23E).

Conclusions:

The addition of propionic acid improved herbicidal efficacy on all weeds, including yellow nutsedge.

Weed Experiment 14: A third experiment was conducted using acrolein with butyric acid, another naturally occurring organic acid of bacterial origin with herbicidal and fungistatic properties.

Materials and Methods:

The test was setup similarly as for Weed experiment 13 but butyric acid was used instead of propionic acid. Pots were uncovered 6 days after treatment and weed counts were taken at 6, 8, 10, and 13 days after treatment.

Results:

Acrolein treatments of 20 and 40 mg acrolein improved large crabgrass control when applied in combination with butyric acid while butyric acid alone at 250 mg resulted in stimulation of large crabgrass (Fig. 24A). All acrolein treatments \leq 60 mg applied with 250 mg butyric acid resulted in better control of morningglory than acrolein applied alone (Fig. 24B). Treatments of butyric acid alone at 250 mg caused only a slight reduction in morningglory (Fig. 24B). All treatments with \geq 80 mg acrolein provided complete control of morningglory (Fig. 24B). Acrolein treatments of 20 and 40 mg resulted in improved sicklepod control when applied with butyric acid than when applied alone (Fig. 24C). Herbicidal efficacy of acrolein treatments of 80 and 100 mg were

improved as well, but the results were significant at the $P=0.10$ level. Butyric acid at 250 mg alone resulted in a slight stimulation of sicklepod (Fig. 24C). When acrolein was applied in combination with butyric acid, there was no improvement in yellow nutsedge control compared to acrolein alone (Fig. 24D). There were fewer weeds in pots treated with 20 and 40 mg acrolein with butyric than in pots treated with 20 and 40 mg acrolein alone (Fig. 24E). Butyric acid did not improve total weed control for high rates of acrolein (Fig. 24E).

Conclusions:

With the exception of yellow nutsedge, lower rates of acrolein were required to control weeds when applied in combination with butyric acid. Butyric acid did not improve yellow nutsedge control. The lowest two rates of acrolein used gained the most herbicidal benefit from the addition of butyric acid.

Combination Experiments with Broad-Spectrum Pesticides: In efforts to reduce the amount of acrolein required to control weeds, a series of experiments were conducted using acrolein in combination with two registered broad-spectrum pesticidal compounds: metam sodium and H-cyanamide.

Weed Experiment 15: In this experiment, metam sodium (Vapam®) was applied in combination with acrolein by drenching into pots with mature yellow nutsedge plants. Metam sodium, a dithiocarbamate, breaks down in the soil to produce methyl-isothiocyanate (MITC) to control weeds, insects, fungi, nematodes, and other plant deleterious microorganisms.

Materials and Methods:

The test was prepared as described for Weed Experiment 2, using Tallassee, AL field soil with five yellow nutsedge tubercles being added before treatment. A 2% acrolein solution was prepared by using 22.22 ml 90% acrolein (Aldrich™) in a final volume of 1000 ml with tap water. A 2% Vapam® (containing 42% metam sodium) solution was made by adding 20 ml Vapam® to tap water and bringing final volume up to 1000 ml. Appropriate amounts of the stock solutions were delivered into plastic cups and the final volume applied per pot was brought up to 100 ml with tap water.

Treatments were drench-applied and 100 ml water alone was applied to the controls.

Metam sodium was applied alone at 6.5, 13, 26, and 39 mg/kg soil. Metam sodium was also applied at the same rates with the addition of 100 mg acrolein per kg soil. Acrolein was applied alone at 100 mg/kg soil. Pots were covered with 1 mil thick plastic bags immediately following drenching and then sealed with rubber bands. Seven replications were used per treatment and two controls were included. Pots were arranged on a greenhouse bench in a randomized complete block design. Pots were uncovered 11 days after treatment and weed counts were taken at 11, 19, and 26 days after treatment.

Results:

In general, all metam sodium plus acrolein treatments had fewer number of nutsedge plants per pot than the metam sodium treatments alone (Fig. 25A); however, this was only significant for the 6.5 and 13 mg rates of metam sodium (Fig. 25A).

Nutsedge was the predominant weed species in this test and the total weed count data are very similar to the nutsedge data alone (Fig. 25B).

Conclusions:

The addition of acrolein improved the herbicidal efficacy of metam sodium and reduced the rates of metam required.

Weed Experiment 16: A second combination tests was setup using metam sodium, only this experiment was conducted to explore post-emergent herbicidal efficacy. Metam sodium and acrolein were applied at the same rates and in the same combinations as they were in the previous experiment onto soil containing yellow nutsedge plants.

Materials and Methods:

Preparation and setup was similar to Weed Experiment 5 using 1 kg pots of Tallassee, AL soil with 5 - 10 yellow nutsedge plants in each one. Stock solutions, treatments, and experimental design were the same as for Weed Experiment 15. Pots were uncovered 4 days after treatment and weed counts were taken at 7, 14, 22, and 29 days after treatment.

Results:

The 6.5 mg rate of metam sodium resulted in better control of yellow nutsedge when applied in combination with acrolein than when applied alone; all other metam sodium treatments were not improved by the addition of acrolein (Fig 26A). Acrolein treatments alone resulted in significant reduction of yellow nutsedge as compared to the controls (Fig. 26A). Any treatment with metam sodium at rates ≥ 26 mg/kg provided complete control of yellow nutsedge (Fig. 26A).

Conclusions:

Herbicidal activity of the lowest rate of metam sodium was improved by the addition of acrolein; however, little improvement was seen with higher rates of metam which provided almost complete control alone.

Weed Experiment 17: A third combination experiment was setup using the broad-spectrum compound H-cyanamide. Ca-cyanamide was the first artificial nitrogen fertilizer and has been used for over a century for pre-plant disinfestations of soil. H-cyanamide, the active ingredient in Ca-cyanamide, is used to defoliate crops and to break bud dormancy in some trees (Dozier *et al.*, 1990; Fallahi *et al.*, 1992). Recent studies show H-cyanamide can be used to control plant pathogenic nematodes and weeds (Rodríguez-Kábana *et al.*, 2003).

Materials and Methods:

The test was prepared as for Weed Experiment 15 using Tallassee, AL field soil with five yellow nutsedge tubercles. A stock 2% acrolein solution was prepared and a stock 2.5% cyanamide solution was prepared using 50 ml of 50% H-cyanamide (Aldrich™) in a 1000 ml final volume with tap-water. Treatments were applied as drench applications using 100 ml final volume per pot; controls were treated with water only. Acrolein was applied alone at 50, 100, 150, and 200 mg/kg soil. Acrolein was also applied at the same rates with the addition of 250 mg cyanamide/kg soil. Cyanamide was applied alone at 250 mg/kg soil. Immediately following drenching, pots were covered with 1 mil thick plastic bags and then sealed with rubber bands. Pots were arranged on a greenhouse bench in a randomized complete block design with two water only controls.

Pots were uncovered 9 days after treatment and weed counts were taken at 9, 15, and 21 days after treatment.

Results:

The only improvement in nutsedge control in the combination treatments was noted in the first weed count with the 100 mg acrolein treatment (Fig. 27A). The only improvement in total weed control was noted in the same count with the same rate (Fig. 27B). No other differences were observed throughout the experiment.

Conclusions:

In general, the addition of 250 mg/kg cyanamide to most of these acrolein treatments did not result in improved nutsedge control.

Weed Experiment 18: A pre-plant experiment was conducted using acrolein and cyanamide. The experiment was designed to explore the relationship between low rates of acrolein and H-cyanamide.

Materials and Methods:

The test was prepared, setup, and arranged as for Weed Experiment 13 using the supplemental “weed pack” with five nutsedge tubercles added to the soil. Treatments were also the same except 250 mg H-cyanamide was used instead of propionic acid. Pots were uncovered 7 days after treatment and weed counts were taken at 7, 14, 10, and 20 days after treatment.

Results:

Any treatment containing cyanamide resulted in excellent control of all weeds throughout the experiment (Figs. 28A, 28B, 28C, 28D, and 28E). All treatments with

250 mg cyanamide improved the herbicidal efficacy of acrolein, but this rate of cyanamide also resulted in complete control of weeds so it was not possible to compare the combinations to acrolein alone. Although the differences were not significant at the $P=0.05$ level, increasing rates of acrolein resulted in reduction of the herbicidal efficacy of cyanamide on large crabgrass, sicklepod, and yellow nutsedge (Figs. 28A, 28C, and 28D).

Conclusions:

The rates of cyanamide used alone controlled all weeds investigated masking any interactions with acrolein.

Combination Experiments with Commercial Herbicides: Results from previous experiments indicate that acrolein was not effective for control of yellow nutsedge at rates < 300 mg/kg. Three experiments were designed to study the effects of acrolein when applied in combination with registered, economical, commercial herbicides.

Weed Experiment 19: This experiment was conducted to determine if lower rates of acrolein could be effective if applied in combination with the nutsedge-specific dithiocarbamate, EPTC (Eptam®). EPTC is typically incorporated into the soil prior to planting at rates 2.2 - 9.5 kg/ha to control weeds in corn, potatoes (*Solanum tuberosum* L.), tomatoes, sugarbeets (*Beta vulgaris* L.), green peas (*Pisum* spp.), pines (*Pinus* spp.), walnuts, almonds, cotton, alfalfa, flax (*Linum usitatissimum* L.), and citrus (*Citrus* spp.)(Senseman, 2007).

Materials and Methods:

The experiment was setup as for Weed Experiment 13 with EPTC in place of propionic acid. Preparation of the 2% stock acrolein solution was previously described. A stock 0.119% EPTC solution was prepared by adding 1.192 ml Eptam® 7EC to 998.808 ml of demineralized water. Treatments were applied by drenching in 100 ml final volume per pot. EPTC was applied at 1, 2, 3, 4, and 5 mg/kg soil; each rate was applied to two sets of replications and all pots were covered. One set of replications received no other treatment, but the other set of replications had been treated with 100 mg acrolein/kg soil 2 days prior to the application of EPTC. Two controls with water only were included. Pots were uncovered 3 days after EPTC treatments. Weed counts were taken at 3, 6, 12, and 24 days after EPTC treatment.

Results:

Large crabgrass was not detected in the first two weed counts and very few were found in the controls by the third count (Fig. 29A). Acrolein alone provided some control of large crabgrass but control was improved with EPTC combinations (Fig. 29A). Jimson weed was also not detected in the first two weed counts; however, by the third weed count jimsonweed was found in all pots with EPTC alone, but not in any of the combination treatments (Fig. 29B). In the first and second weed counts all pots treated with EPTC alone had fewer morningglory plants per pot than the controls, but all pots treated with acrolein had virtually none (Fig. 29C). Acrolein alone resulted in complete control of morningglory (Fig. 29C). The herbicidal efficacy of EPTC on sicklepod was improved by the addition of acrolein in the first two weed counts; however, results were inconsistent in the final two weed counts (Fig. 29D). All treatments containing EPTC

resulted in excellent control of yellow nutsedge throughout the experiment; acrolein alone stimulated nutsedge (Fig. 29E). Acrolein did not improve the efficacy of EPTC for control of yellow nutsedge (Fig. 29E). There were fewer total weeds in all pots treated with EPTC plus acrolein than in those treated with EPTC alone (Fig. 29F).

Conclusions:

Excellent weed control was noted with acrolein and EPTC combinations; lower rates of both compounds were required to control weeds. Weeds that were not controlled well by EPTC alone were controlled by the combinations; conversely, weeds not controlled well by acrolein alone were controlled by the combinations.

Weed Experiment 20: An experiment was conducted using acrolein in combination with *s*-metolachlor (Dual Magnum®), a common agricultural herbicide with good yellow nutsedge efficacy. *S*-metolachlor was used in this trial to help reduce the rates of acrolein required to control weeds, specifically yellow nutsedge. *S*-metolachlor is typically applied prior to planting to control nutsedge, foxtails, many annual grasses, and other weeds in corn, cotton, peanuts (*Arachis hypogaea* L.), turfgrass, nursery crops, and tomatoes (Senseman, 2007; Gilreath and Santos, 2005).

Materials and Methods:

The test was setup as described for Weed Experiment 19 with *s*-metolachlor (Dual Magnum®) in place of EPTC. Preparation of the 2% stock acrolein solution was previously described and a 0.0547% stock *s*-metolachlor solution was prepared by adding 1.095 ml Dual Magnum® (7.62 lbs active ingredient/gallon) to 998.905 ml of demineralized water. *S*-metolachlor was applied at 1, 2, 3, 4, and 5 mg/kg soil alone, and

the same rates were applied in combination with 100 mg acrolein/kg soil. Acrolein was also applied alone at 100 mg/kg soil. Pots were uncovered 6 days after treatment and weed counts were taken at 6, 12, 20, and 27 days after treatment.

Results:

Large crabgrass was not detected in the first weed count; however, it was noted in subsequent counts when all treated pots had less large crabgrass than the controls (Fig. 30A). Acrolein alone provided some control of large crabgrass, but added little to the efficacy of *s*-metolachlor alone which completely controlled the weed at all rates used (Fig. 30A). Through the duration of the test, *s*-metolachlor alone did not control morningglory while all treatments containing acrolein provided excellent control (Fig. 30B). The addition of 100 mg acrolein improved the efficacy of *s*-metolachlor against sicklepod in the first and second weed counts; however, results were inconsistent in the final two counts (Fig. 30C). Acrolein alone stimulated yellow nutsedge and only improved control for the 1, 1.5, and 2 mg rates of *s*-metolachlor in the final two nutsedge counts (Fig. 30D). The addition of acrolein to the *s*-metolachlor treatments resulted in fewer total weeds than the *s*-metolachlor treatments alone (Fig. 30E). Combination treatments with *s*-metolachlor improved total weed control (Fig. 30E).

Conclusions:

With the exception of nutsedge, applications of acrolein in combination with *s*-metolachlor resulted in improved herbicidal activity compared with either compound alone.

Weed Experiment 21: A third experiment was conducted to evaluate herbicidal efficacy of acrolein when applied with in combination with halosulfuron (Sanda®).

Halosulfuron is labeled for use in agriculture and is known to provide selective control of sedges and broadleaves in corn, grain sorghum (*Sorghum* spp.), sugarcane, turfgrasses, peppers, and tomatoes (Norsworthy *et al.*, 2005; Senseman, 2007). This herbicide is generally applied at 35 - 70 g active ingredient per ha, and can be applied prior to planting or for post-emergent weed control. This experiment was designed to determine the efficacy of the two compounds applied in combination.

Materials and Methods:

The experiment was setup similarly to Weed Experiment 19 with the exception that halosulfuron (Sanda®) was used in place of EPTC. Preparation of the 2% stock acrolein solution was previously described and a 0.1 mg/ml halosulfuron solution was prepared by first dissolving 1.33 grams of Sandea® (75% active ingredient) into 1 L demineralized water and then adding 10 ml of this solution to 990 ml of demineralized water. Sandea® was applied at 0.01, 0.025, 0.05, 0.075, and 0.1 mg/kg soil alone, and the same rates were applied in combination with 100 mg acrolein/kg soil. Acrolein was also applied alone at 100 mg/kg soil. Pots were uncovered 5 days after treatment and weed counts were taken 5, 10, 18, and 25 days after treatment.

Results:

No large crabgrass was noted in the first weed count. In the next three weed counts, all treatments that contained acrolein had fewer large crabgrass plants per pot than the controls or those treated with halosulfuron alone (Fig. 31A). All pots treated with halosulfuron alone had similar number of morningglory plants per pot as the

controls, while all pots treated with acrolein had virtually none for all four weed counts (Fig. 31B). The addition of acrolein to halosulfuron improved control of sicklepod in the first two counts; however, results were inconsistent in the final two counts (Fig. 31C). Halosulfuron treatments resulted in excellent yellow nutsedge control with all rates used; combinations with acrolein did not improve the efficacy of halosulfuron to control the weed (Fig. 31D). There were fewer weeds in pots treated with the combination treatments than in any pot treated with halosulfuron alone (Fig. 31E).

Conclusions:

Acrolein and halosulfuron combination treatments had much fewer weeds than either compound alone. Acrolein controlled the large crabgrass and morningglories which halosulfuron did not control well; conversely, halosulfuron controlled the nutsedge and acrolein did not.

Microplot Experiments: A series of microplot experiments were conducted to test the herbicidal efficacy of acrolein.

Weed Experiment 22: This microplot experiment was conducted to investigate the efficacy of drench-applied acrolein compared to drench-applied metam sodium.

Materials and Methods:

Microplot description and material and methods were described in Nematode Test 10. Acrolein was drench-applied at rates 50, 75, 100, 125, and 150 mg/kg soil (1 mg/kg soil = 1 kg/ha) in 3.785 L final volume. Metam sodium (Vapam®) was applied at the recommended rate of 127.8 mg/kg soil (92 L/ha or 60 gallons per acre) and a water-

treated control was included. Plots were uncovered 5 days after treatment and weed survival ratings were made from digital photographs taken at 5, 8, and 15 days after treatment (rating scale 0 - 5; 5= no weed control, 0= complete weed control). Details of the setup and experimental design were described in the previous chapter.

Results:

All treated plots had fewer weeds than the control plots in all weed survival ratings (Fig. 32A). Plots treated with 150 mg acrolein had fewer weeds than plots treated with 50 and 75 mg acrolein in 8 and 15 days after treatment (Fig. 32A). The metam sodium treatment had fewer weeds than all acrolein-treated plots except the 150 mg rate in the first rating; metam sodium had fewer weeds than plots treated with 50 and 75 mg acrolein in the second and third ratings (Fig. 32A).

Conclusions:

Rates of acrolein ≥ 150 mg/kg soil alone did provide weed control comparable to metam sodium at 127.8 mg/kg soil (92 L/ha or 60 gallons per acre).

Weed Experiment 23: This microplot experiment was conducted to determine the relationship between the use of plastic cover and herbicidal efficacy of acrolein. The chemical was compared to metam sodium applied with and without plastic cover.

Materials and Methods:

Microplots and experimental design were as for Weed Experiment 22. Acrolein was drench applied at 100 and 200 mg/kg soil (100 and 200 kg/ha) in 3.785 L final volume per plot. Metam sodium (Vapam®) was applied at the recommended rate of 127.8 mg/kg soil (92 L/ha or 60 gallons per acre) and a water-treated control was

included. All three chemical treatments were applied to two plots per replication; one plot was not covered after treatment and the other was covered with a plastic bag and sealed with a large rubber band. Plots were uncovered 4 days after treatment and weed survival ratings were made from digital photographs taken at 4, 16, 27, and 42 days after treatment (rating scale 0 - 5; 5= no weed control, 0= complete weed control).

Results:

All treated plots had fewer weeds than the controls throughout the experiment (Fig. 33A). Covered plots had fewer weeds than plots with no cover (Fig. 33A). In all three ratings, plots treated with metam sodium + cover had fewer weeds than plots treated with 100 mg acrolein + cover; those treated with metam sodium + cover had similar weed survival ratings as those treated with 200 mg acrolein + cover (Fig. 33A). In the first and third weed ratings, 200 mg acrolein treatments without cover resulted in fewer weeds than plots treated with 100 mg acrolein without cover (Fig. 33A). Plots treated with 200 mg acrolein + cover had fewer weeds than plots treated with 100 mg acrolein + cover in all three weed survival ratings (Fig. 33A).

Conclusions:

All treatments benefited from the use of plastic cover.

Weed Experiment 24: The third microplot experiment was conducted to determine the herbicidal efficacy of acrolein when applied as a drench in combination with metam sodium.

Materials and Methods:

Metam sodium (Vapam®) was applied alone at 21.3, 42.6, and 63.9 mg/kg soil (15, 31, and 46 L/ha or 10, 20, and 30 gallons per acre). These same treatments were also applied in combination with acrolein at 50 mg/kg soil (50 kg/ha). Acrolein was applied alone at 50 mg/kg soil and a water-treated control was included. All plots were covered with plastic bags and sealed with large rubber bands. Experiment was as described for Weed Experiment 22. Plots were uncovered 5 days after treatment and weed survival ratings were made from digital photographs taken at 5, 22, and 37 days after treatment (rating scale 0 - 5; 5= no weed control, 0= complete weed control).

Results:

In the first weed rating, all treated plots had fewer weeds than the controls (Fig. 34A). Plots treated with 42.6 and 63.9 mg/kg of metam sodium alone had fewer weeds than plots treated with 42.6 and 63.9 mg of metam sodium + 50 mg acrolein (Fig. 34A). By the second and third ratings there were no differences between the 50 mg acrolein alone and the controls (Fig. 34A). Also in the final two ratings, there were no differences between plots treated with any rate of metam sodium alone and those same rates of metam sodium + 50 mg acrolein (Fig. 34A). In all three ratings, plots treated with 63.9 mg/kg of metam sodium had fewer weeds than those treated with 21.3mg/kg, with or without acrolein (Fig. 34A).

Conclusions:

Tank mixing acrolein and metam sodium reduces the herbicidal activity of the compounds. These treatments should not be applied together.

D. DISCUSSION

Direct applications of acrolein controlled large crabgrass and pigweed at rates \leq 200 mg acrolein; however, 300 - 450 mg of acrolein was required to control yellow nutsedge. Low rates of acrolein did result in increase of yellow nutsedge. Controlling sedges is very important in production systems that use plastic mulch to cover the beds because sedges are notorious for puncturing the plastic which allows weeds to overtake the beds. Drench applications using aqueous solutions of acrolein proved more effective for controlling weeds than direct applications of the chemical. The final volume of the drench did not affect herbicidal efficacy as much as the rates of acrolein did. Drench applications of \geq 200 mg acrolein to yellow nutsedge plants resulted in excellent control. These rates caused no phytotoxicity to plants grown in the pots after treatment, indicating that these rates could be used to disinfest agricultural fields prior to planting. Although they were not compared in any of these experiments, effective rates of drench-applied acrolein are comparable to commonly used rates of methyl bromide (150 - 225 kg/ha). Unlike MITC generators, acrolein is not dependent on soil microorganisms to activate the compound; it is active on contact like methyl bromide.

When used to fumigate soil, acrolein provided excellent herbicidal efficacy, controlling most weeds at rates \geq 200 mg/kg soil, and controlling yellow nutsedge with 200 - 250 mg. *Trichoderma* spp. were observed on the soil surface in many of the pots fumigated with acrolein. Increasing soil populations of this group of fungi can be considered as improving the soil microflora; *Trichoderma* spp. are known to compete with soil-borne pathogenic fungi and are used for biological control. When aqueous solutions of acrolein were used to fumigate soil, a reduction in efficacy was observed as

the proportion of water in the solution increased. Similar to the discussion about the amount of water in drench solutions, the amount of acrolein in the solution had more effect on herbicidal efficacy than the amount of water in the fumigant solution. Results indicate that when using acrolein in aqueous solution to fumigate soil, the least amount of water may be the most effective. The moisture of the soil may also have a significant impact on the efficacy of fumigating soils with acrolein. Acrolein treatments did not control weeds in saturated soils at the same rate that they were effective in soils at ½ field capacity. Since acrolein can be used to fumigate soil, it is possible that the compound can be applied with a tractor driven shank-injection apparatus, similar to those used for methyl bromide treatments. This would allow growers to use acrolein instead of methyl bromide with few modifications of their current equipment and production systems; other alternatives to methyl bromide require specialized equipment and techniques (Culpepper *et al.*, 2006).

When acrolein was used as a drench application to control multiple pest pressures, acrolein provided good control of large crabgrass and morningglory at rates > 100 mg/kg soil; however, twice the amount of acrolein was required to control yellow nutsedge. The compound proved effective at controlling a broad-spectrum of pathogens and weeds. Morningglory is the most sensitive to the compound of those studied, less sensitive are large crabgrass and sicklepod, and yellow nutsedge is the most tolerant.

The herbicidal efficacy of acrolein was improved when it was applied in combination with propionic acid and butyric acid. These are naturally occurring products of bacterial anaerobic fermentation. The addition of propionic acid improved the herbicidal efficacy of acrolein treatments on every weed studied, including yellow

nutsedge. Data suggest that drench applications of acrolein in combination with propionic acid may have better herbicidal efficacy in the field than that of acrolein alone. Butyric acid also improved the herbicidal efficacy of acrolein treatments, but it did not improve control of yellow nutsedge. Butyric acid may be useful for reducing the rates of acrolein required to control some agricultural weeds. The addition of glycerin did not improve the herbicidal efficacy of acrolein treatments, but it did result in an increase of *Trichoderma* spp. on the soil surface. These are very positive results of acrolein treatments since *Trichoderma* spp. are known to suppress diseases and improve plant growth (Papavizas, 1985; Windham and Elad, 1986).

Excellent weed control was noted with acrolein in combination with EPTC, *s*-metolachlor, and halosulfuron. When applied in combination with any of these three herbicides, lower rates of both compounds were required to control weeds. Weeds, such as morningglory and large crabgrass that were not controlled well by the herbicides alone, were controlled by the combinations with acrolein. Conversely, weeds not controlled well by acrolein alone, like nutsedge and sicklepod, were controlled by the combinations with the herbicides. These combinations may be able to provide herbicidal control in agricultural fields and significantly reduce the amount of acrolein that would be required to ≤ 100 mg/kg (100 kg/ha or 200 lbs/acre). This is less than the typical 150 - 225 kg of methyl bromide used to treat a hectare. Like methyl bromide, acrolein treatments may be most effective if applied in combination with other pesticides; methyl bromide is normally applied in combination with chloropicrin to help control organisms, like many fungi, that methyl bromide does not control alone.

Acrolein improved the herbicidal efficacy of metam sodium in the greenhouse and reduced the rates of metam sodium required for control. It was also determined that the two compounds are not compatible for tank-mixing, and tank-mixing will reduce herbicidal efficacy of both compounds. If acrolein and metam sodium are to be used as a combination to treat soil, their applications should be made separately. Drench applications in microplots required ≥ 400 mg acrolein per kg soil to result in weed control similar to metam sodium at 127.8 mg/kg soil. Acrolein has herbicidal efficacy when drench-applied without using plastic cover, but it is more effective at low rates when plastic cover is used. Like methyl bromide, the volatile properties of acrolein require that some means of retaining the compound in the soil be used, like plastic mulch, for it to be most effective.

Acrolein has proved to be an effective aquatic herbicide (Anderson and Dechoretz, 1982; Bentivegna and Fernandez, 2003; Bentivegna *et al.*, 2005). Results of this research suggest its potential for use in agriculture as a pre- and post-emergent herbicide. The effective herbicidal rates of acrolein are not only within an acceptable range for agricultural uses, but they generally result in improved and healthier plants. Combinations of acrolein with organic acids, certain herbicides, and other broad-spectrum pesticides can be used to increase herbicidal efficacy of acrolein at low rates.

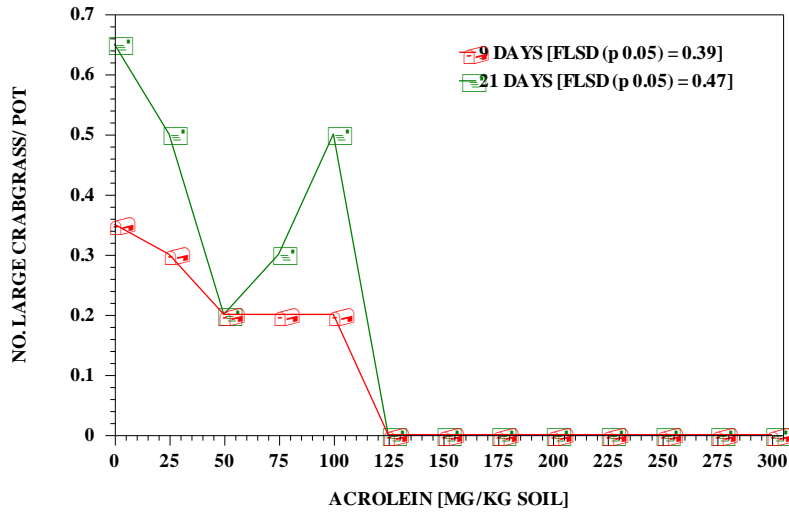


Figure 11A: Number of large crabgrass (*Digitaria sanguinalis*) plant per pot (Weed Experiment 1).

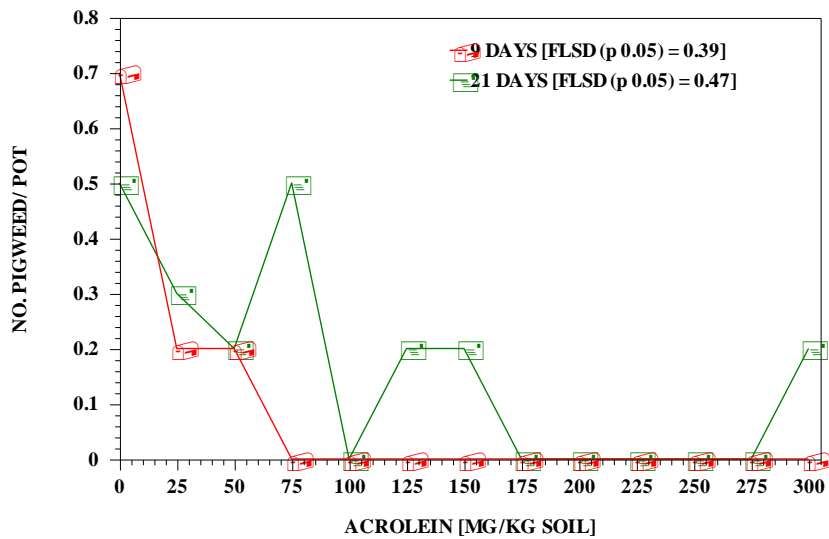


Figure 11B: Number of pigweed (*Amaranthus* spp.) per pot (Weed Experiment 1).

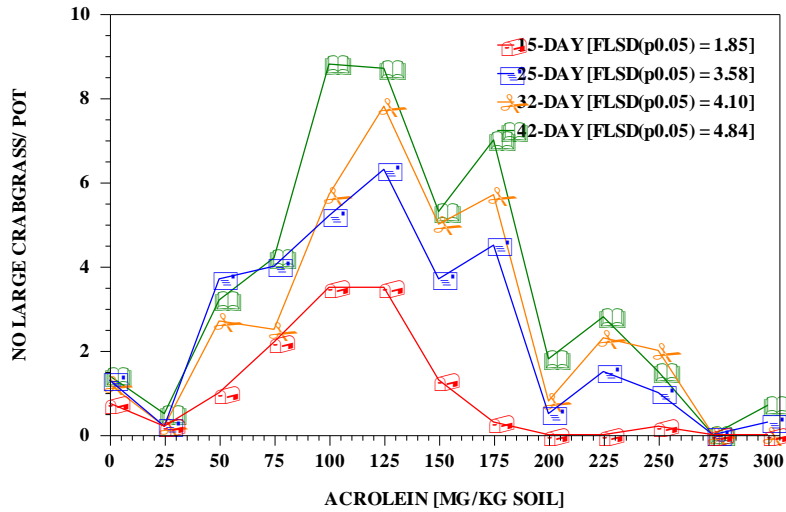


Figure 12A: Number of large crabgrass (*Digitaria sanguinalis*) plants per pot (Weed Experiment 2).

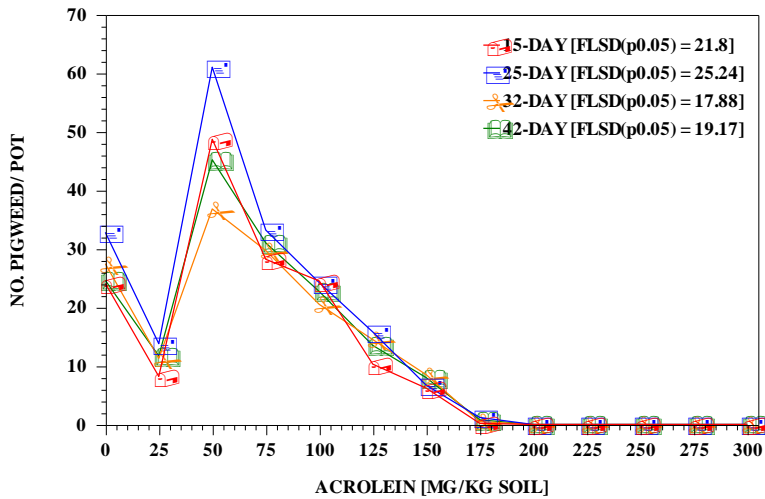


Figure 12B: Number of pigweed (*Amaranthus* spp.) plants per pot in four weed counts (Weed Experiment 2).

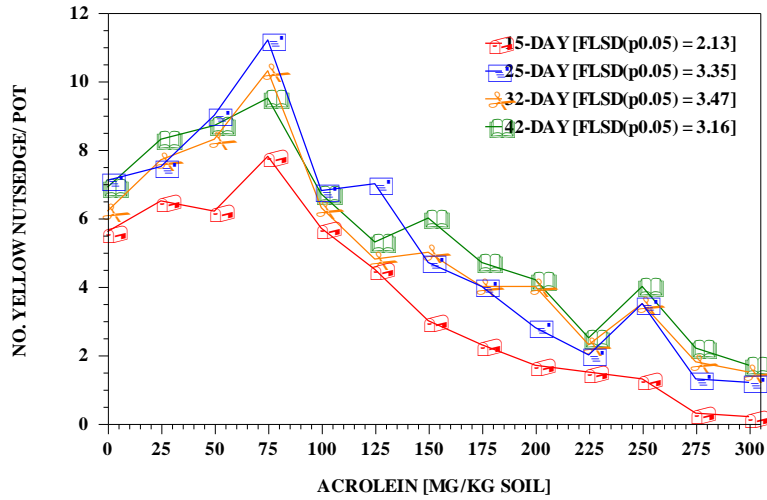


Figure 12C: Number of yellow nutsedge (*Cyperus esculentus*) plants per pot in four weed counts (Weed Experiment 2).

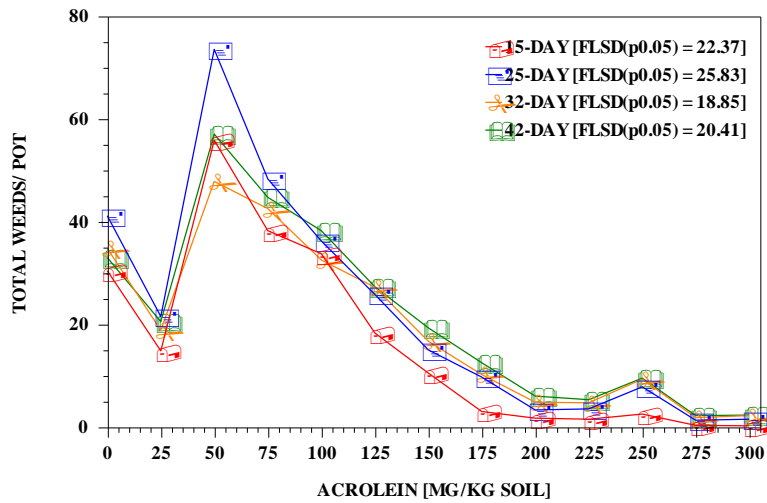


Figure 12D: Total number of weeds per pot in four weed counts (Weed Experiment 2).

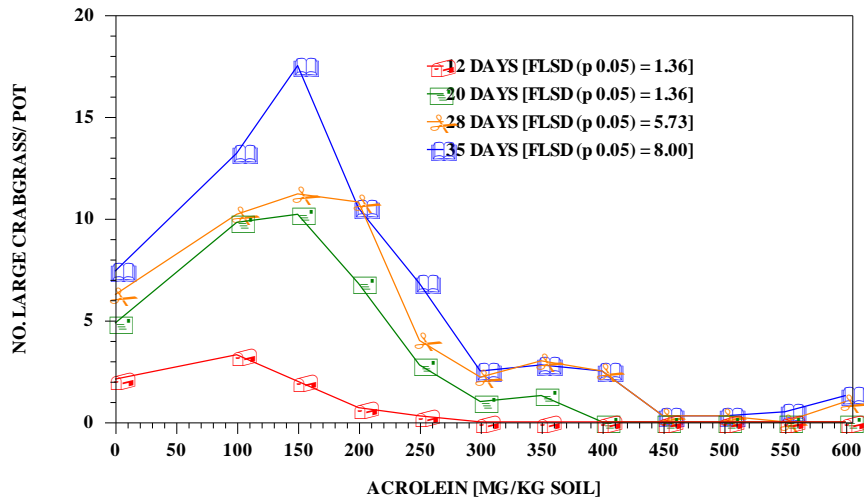


Figure 13A: Number of large crabgrass (*Digitaria sanguinalis*) plants per pot in four weed counts (Weed Experiment 3).

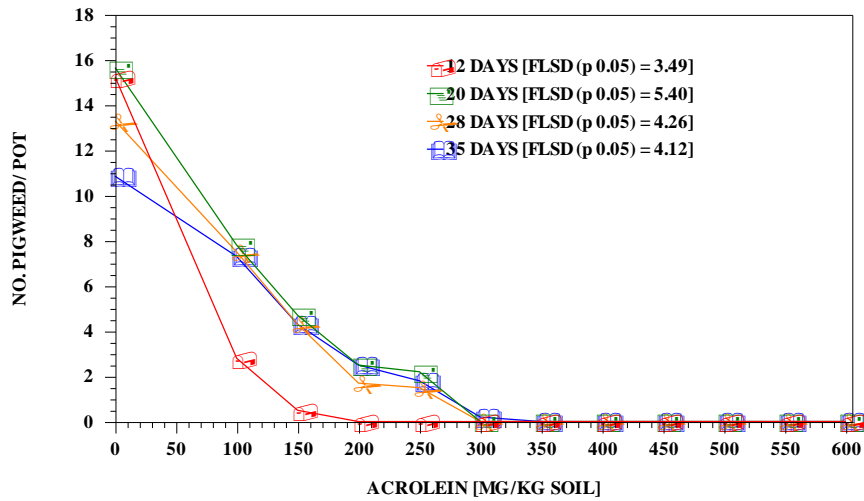


Figure 13B: Number of pigweed (*Amaranthus* spp.) plants per pot in four weed counts (Weed Experiment 3).

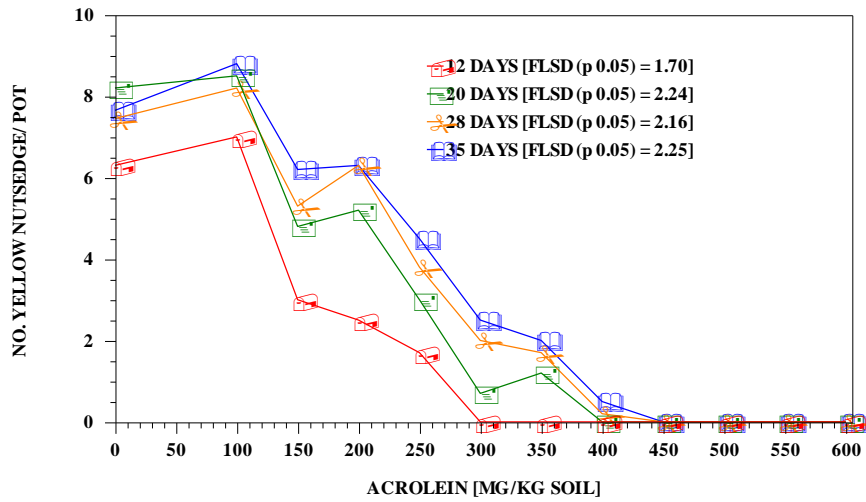


Figure 13C: Number of yellow nutsedge (*Cyperus esculentus*) plants per pot in four weed counts (Weed Experiment 3).

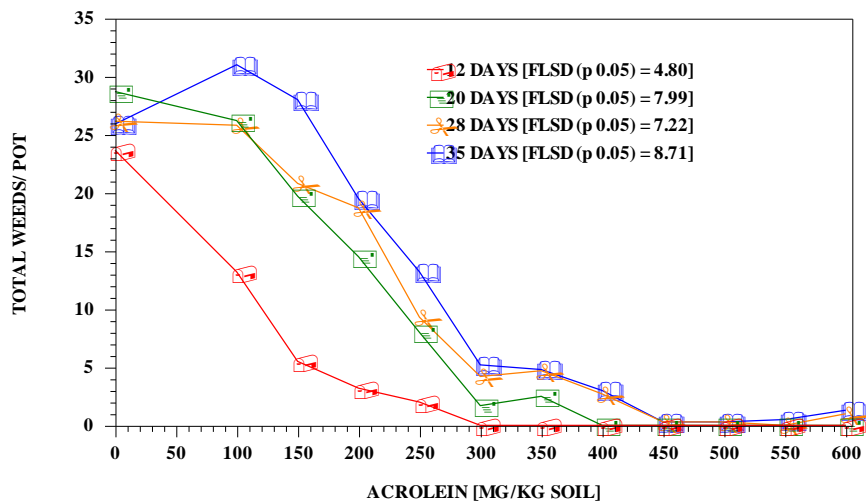


Figure 13D: Total number of weeds per pot in four weed counts (Weed Experiment 3).

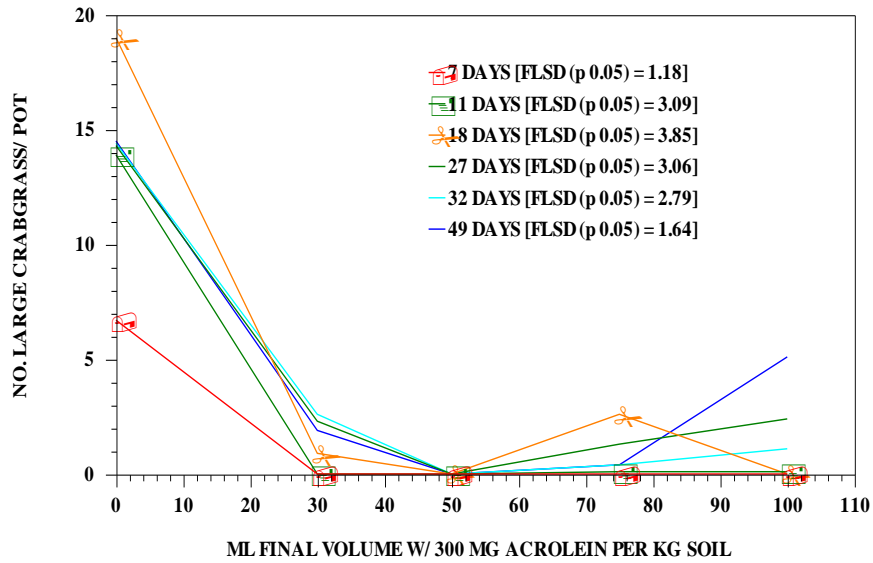


Figure 14A: Number of large crabgrass plants per pot in six weed counts (Weed Experiment 4).

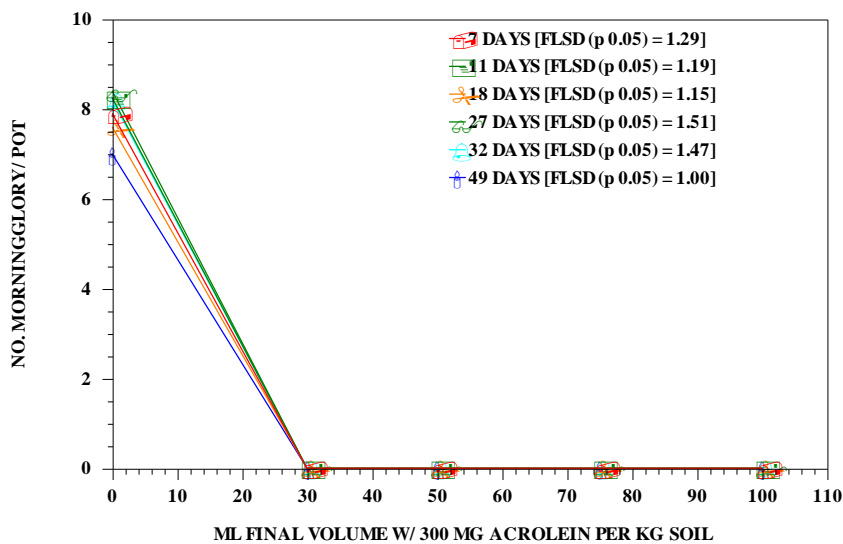


Figure 14B: Number of morningglory plants per pot in six weed counts (Weed Experiment 4).

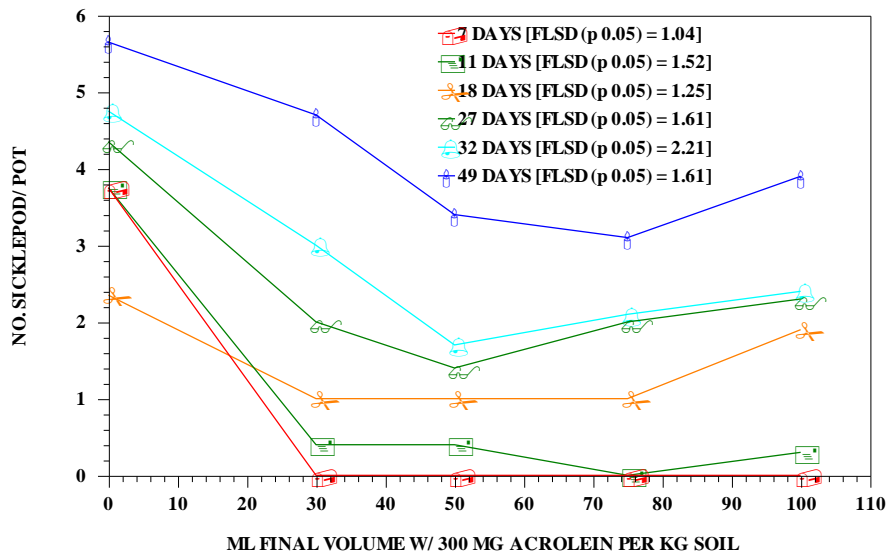


Figure 14C: Number of sicklepod plants per pot in six weed counts (Weed Experiment 4).

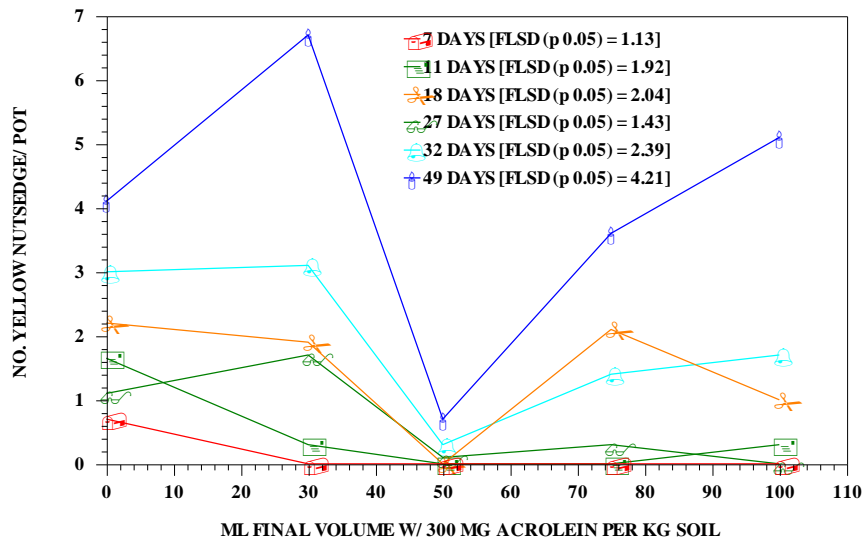


Figure 14D: Number of yellow nutsedge plants per pot in six weed counts (Weed Experiment 4).

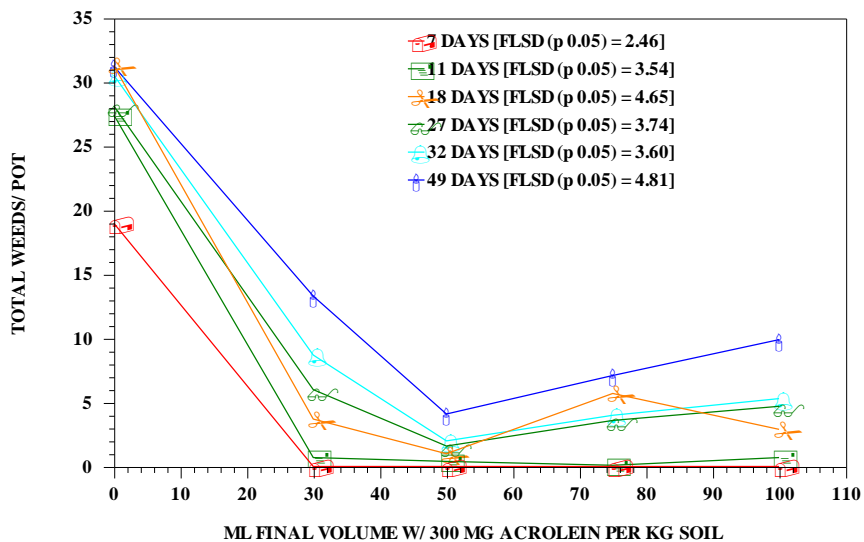
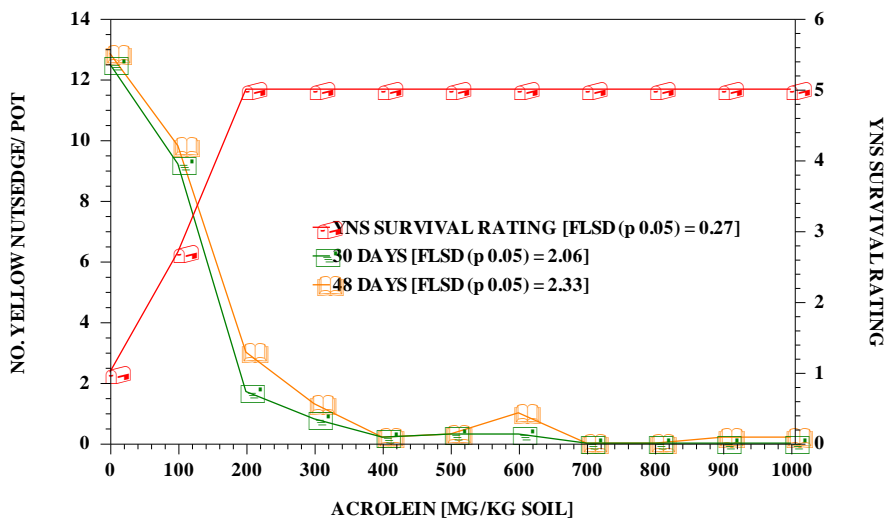


Figure 14E: Total number of weeds per pot in six weed counts (Weed Experiment 4).



SURVIVAL RATING- 5= ALL DEAD, 1= ALL GREEN

Figure 15A: Yellow nutsedge survival rating and number of plants in two weed counts (Weed Experiment 5).

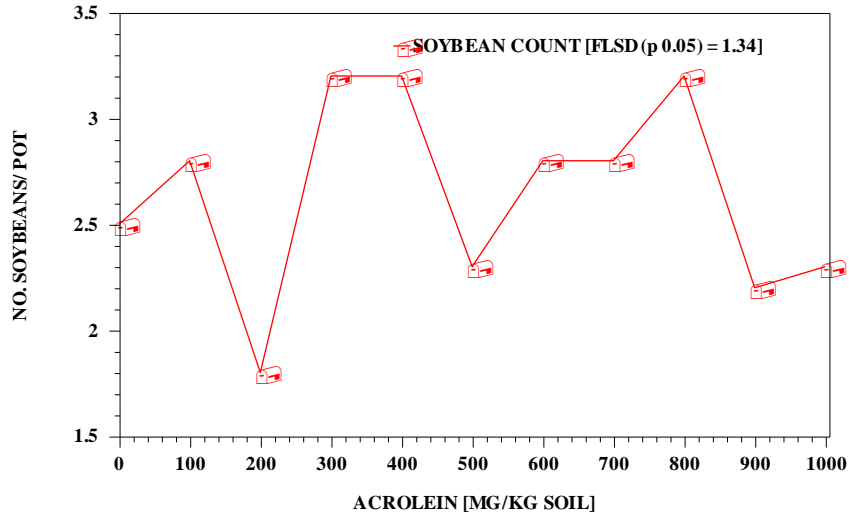
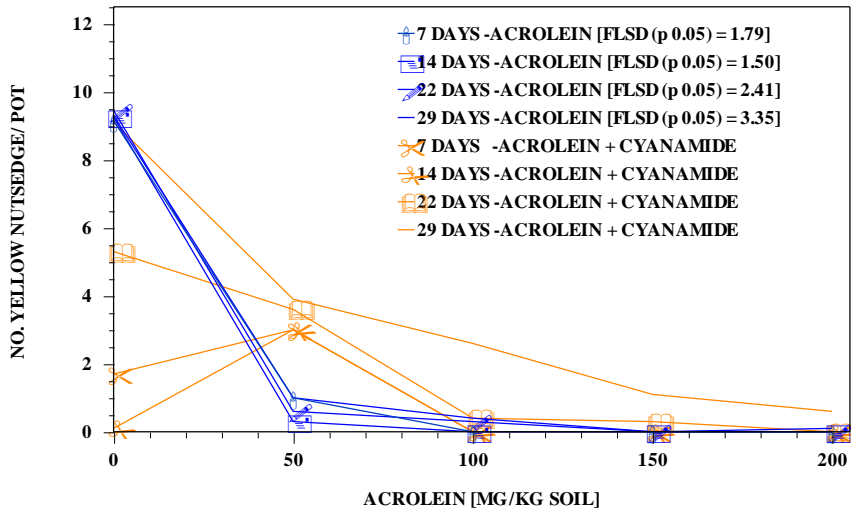


Figure 15B: Number of "Hutcheson" soybean plants (Weed Experiment 5)



Cyanamide applied at 250 mg in all acrolein cyanamide mixtures.

Figure 16A: Number of yellow nutsedge (*Cyperus esculentus*) per pot in four counts (Weed Experiment 6).

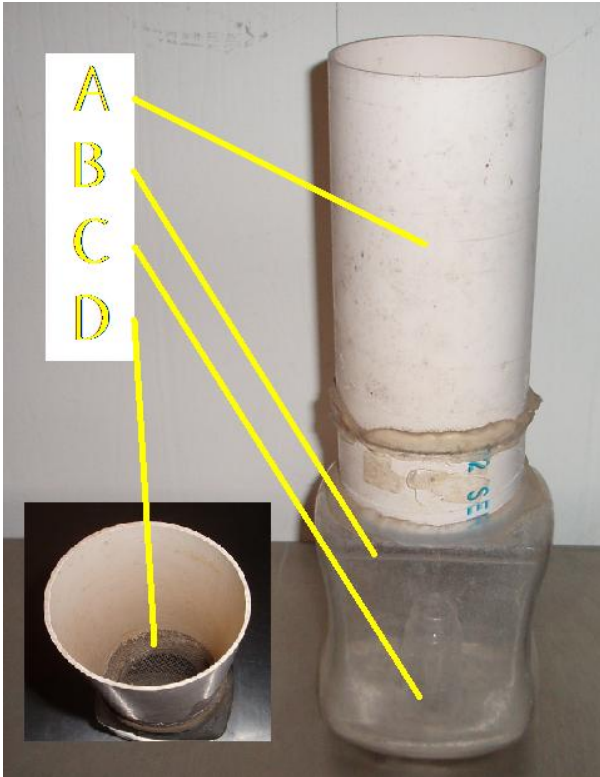


Figure 17A: Fumigation Chamber: A- area for soil (pot), B- fumigant air-space, C- area for fumigant, D- screen in bottom of pot (Weed Experiment 7).

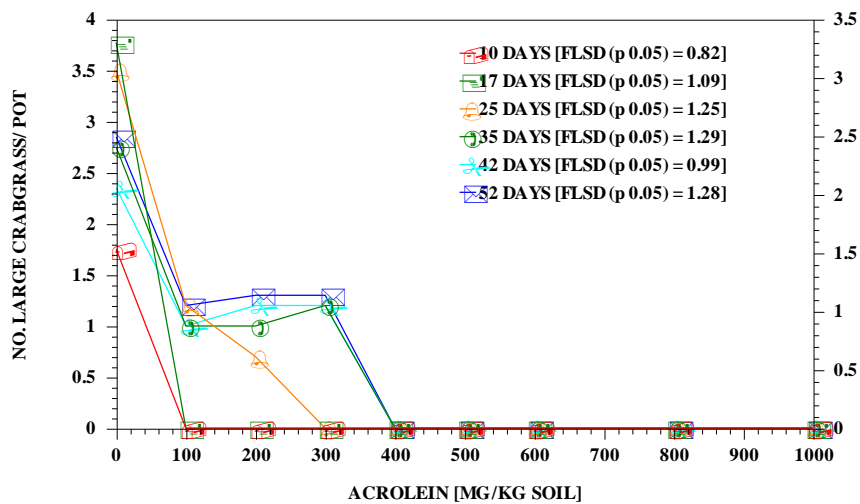


Figure 17B: Number of large crabgrass (*Digitaria sanguinalis*) plants per pot in six weed counts (Weed Experiment 7).

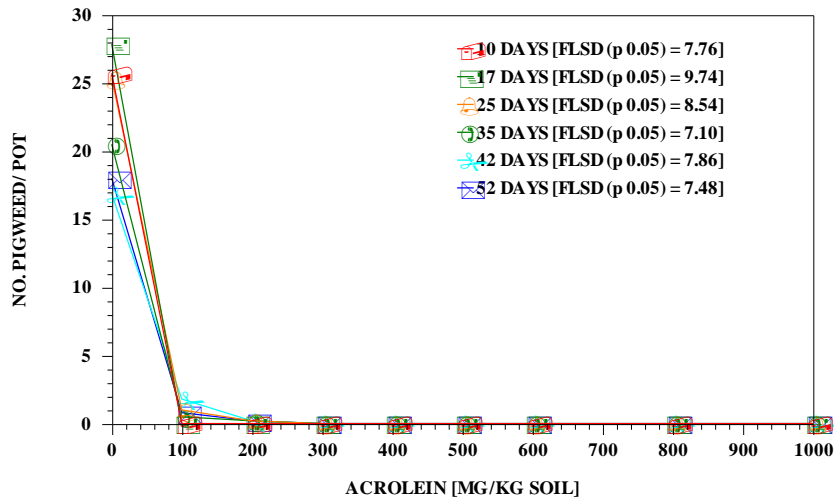


Figure 17C: Number of pigweed (*Amaranthus* spp.) plants per pot in six weed counts (Weed Experiment 7)

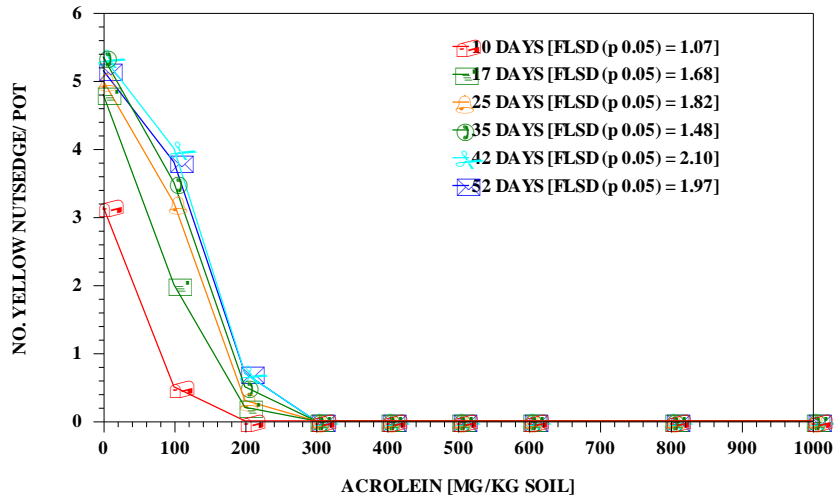


Figure 17D: Number of yellow nutsedge (*Cyperus esculentus*) plants per pot in six weed counts (Weed Experiment 7).

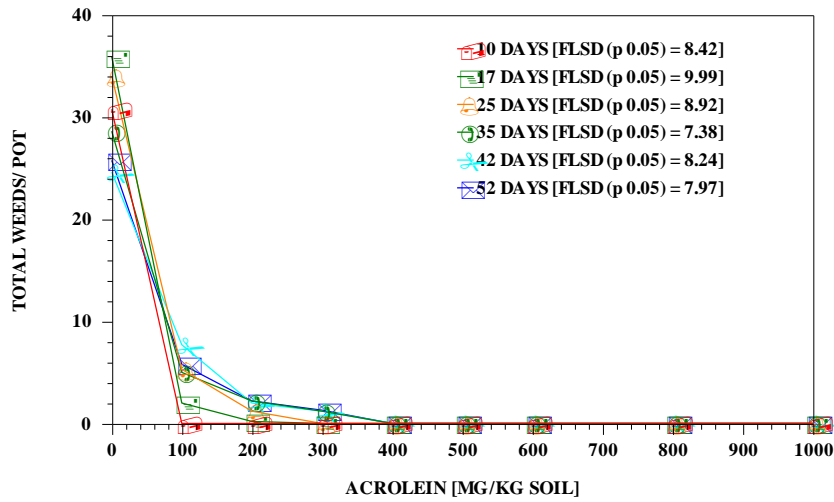


Figure 17E: Total number of weeds per pot in six weed counts (Weed Experiment 7).

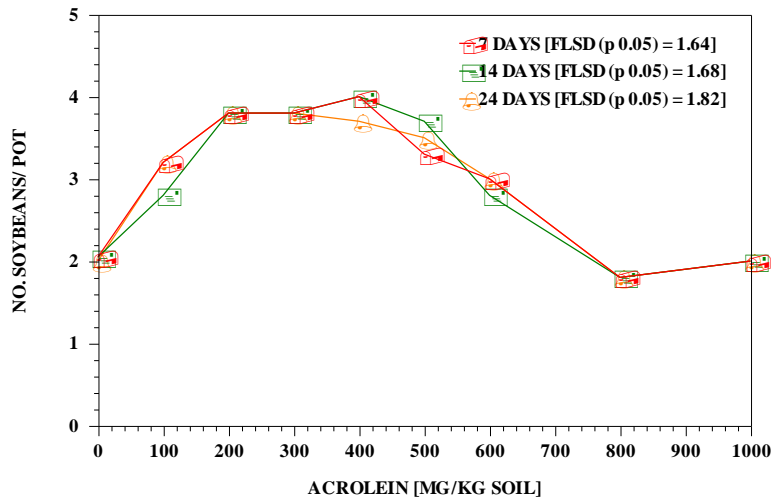


Figure 17F: Number of "Hutcheson" soybeans emerging per pot in three counts (Weed Experiment 7).

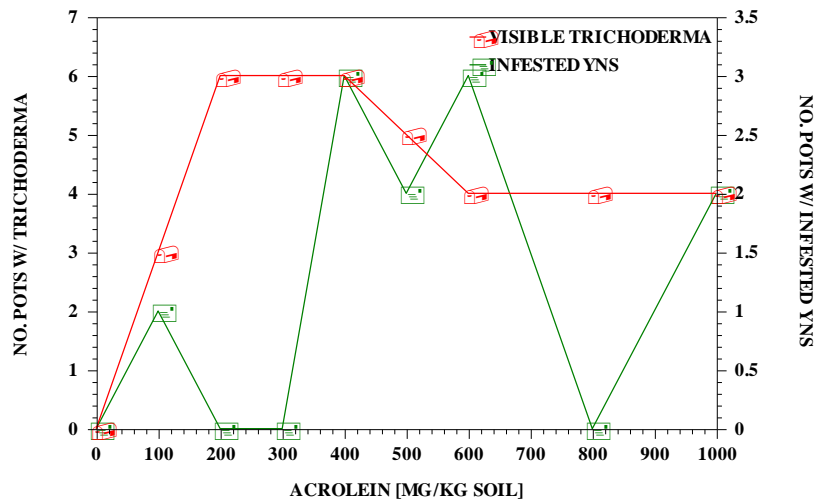


Figure 17G: Number of pots with *Trichoderma* on the soil surface or infesting tubercles (Weed Experiment 7).

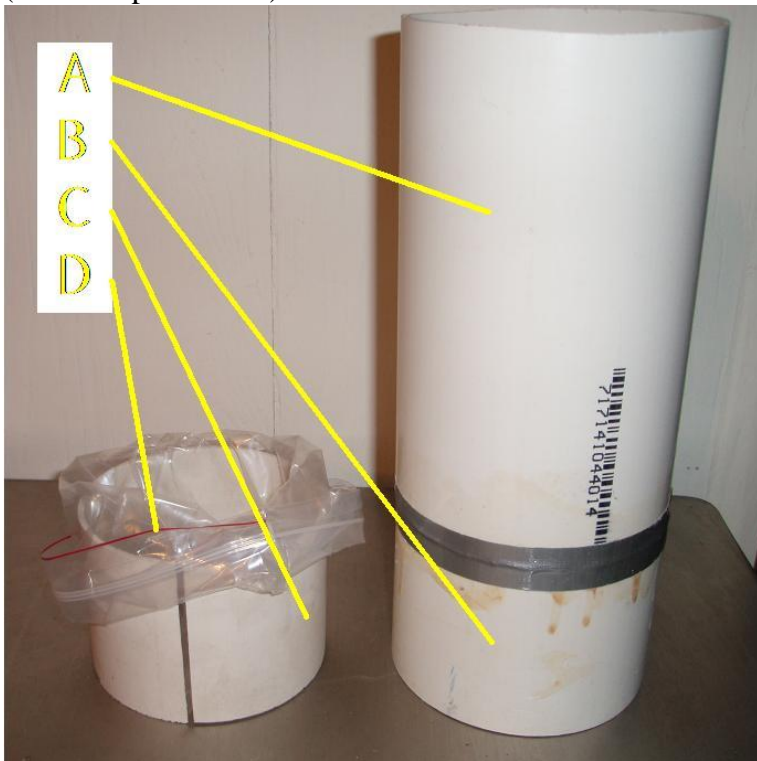


Figure 18A: Fumigation Pot: A- upper portion to contain soil (pot), B- lower area for fumigant ring, C- basal fumigation ring, D- bag to contain fumigant (Weed Experiment 8).



Figure 18B: Fumigation pot being slipped over basal fumigation ring and view through top of pot (Weed Experiment 8).

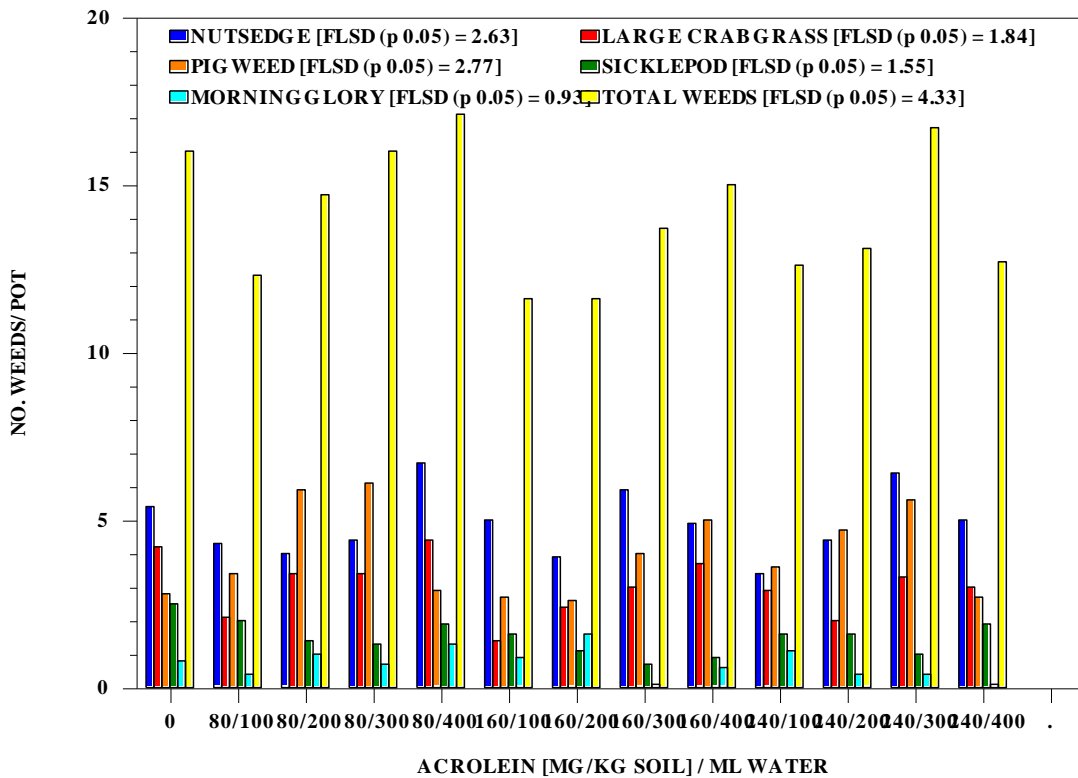


Figure 18C: Weed data summarized for yellow nutsedge, large crabgrass, pigweed, sicklepod, morningglory, and total weeds (Weed Experiment 8).

Treatment	mg Acrolein/ kg soil	ml water in chamber	Yellow Nutsedge	Large Crabgrass	Pigweed	Sicklepod	Morning- glory	Total Weeds
1. Control	0	0	5.4	4.2	2.8	2.5	0.8	16.0
2. 2% Acrolein	80	100	4.3	2.1	3.4	2.0	0.4	12.3
3.	80	200	4.0	3.4	5.9	1.4	0.1	14.7
4.	80	300	4.4	3.4	6.1	1.3	0.7	16.0
5.	80	400	6.7	4.4	2.9	1.9	1.3	17.1
6.	160	100	5.0	1.4	2.7	1.6	0.9	11.6
7.	160	200	3.9	2.4	2.6	1.1	1.6	11.6
8.	160	300	5.9	3.0	4.0	0.7	0.1	13.7
9.	160	400	4.9	3.7	5.0	0.9	0.6	
10.	240	100	3.4	2.9	3.6	1.6	1.1	
11.	240	200	4.4	2.0	4.7	1.6	0.4	13.1
12.	240	300	6.4	3.3	5.6	1.0	0.4	16.7
13.	240	400	5.0	3.0	2.7	1.9	0.1	12.7
LSD (P=0.05) = 2.63			1.84	2.77	1.55	0.93	4.33	

Table 2: Treatment data and weed data summary for yellow nutsedge, large crabgrass, pigweed, sicklepod, morningglory, and total weeds (Weed Experiment 8).

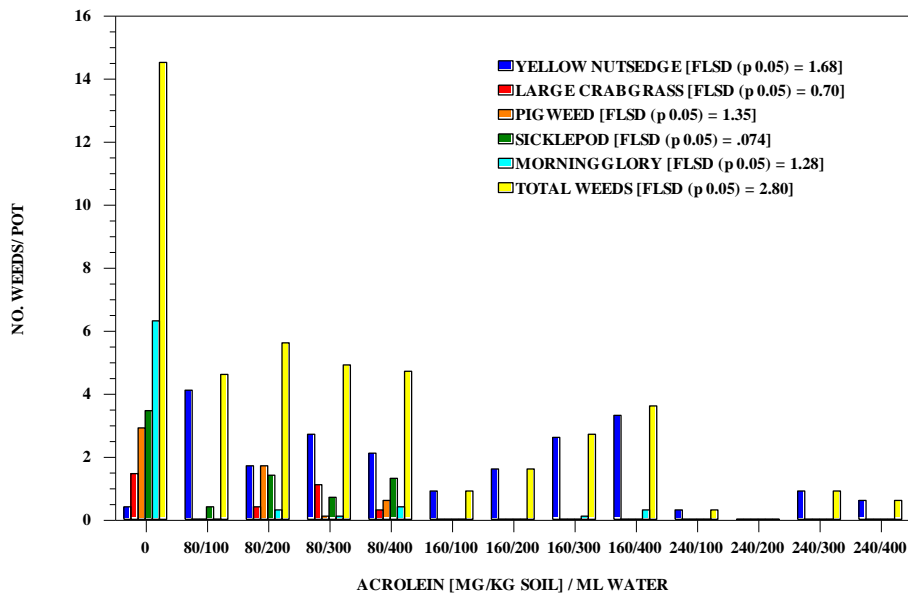


Figure 19A: Summary graph of all weed data and all treatments (Weed Experiment 9).

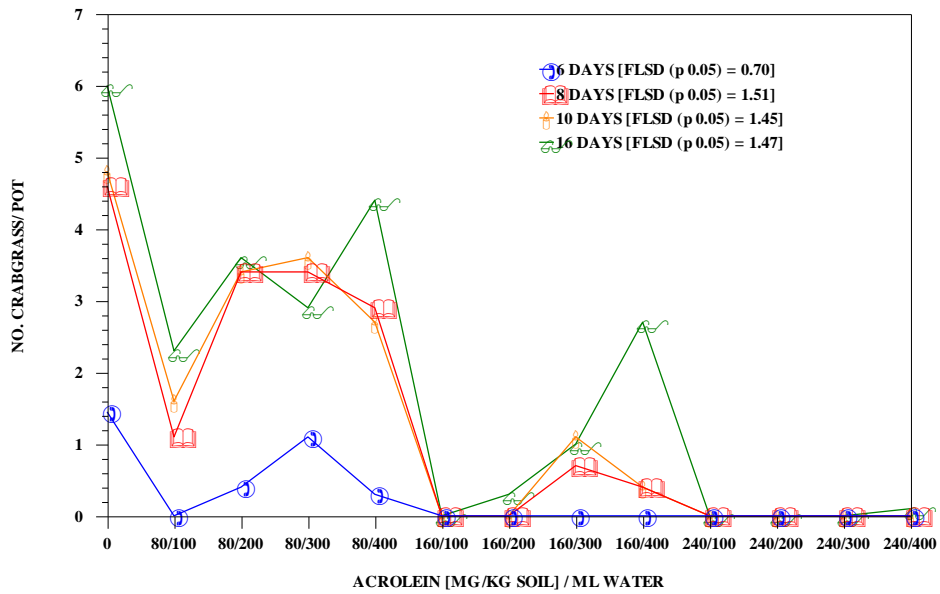


Figure 19B: Number of large crabgrass plants per pot for each in four weed counts (Weed Experiment 9).

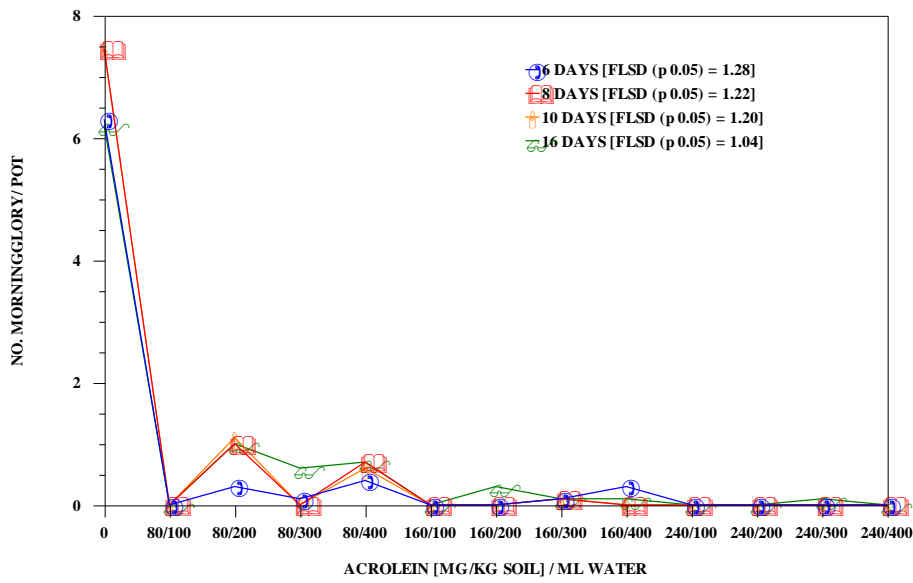


Figure 19C: Number of morningglory plants per pot in four weed counts (Weed Experiment 9).

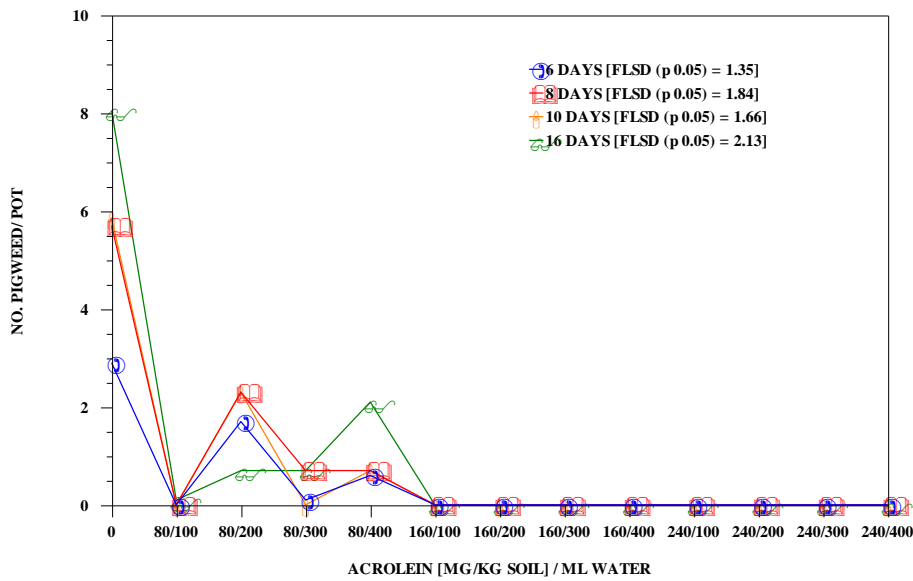


Figure 19D: Number of pigweed plants per pot in four weed counts (Weed Experiment 9).

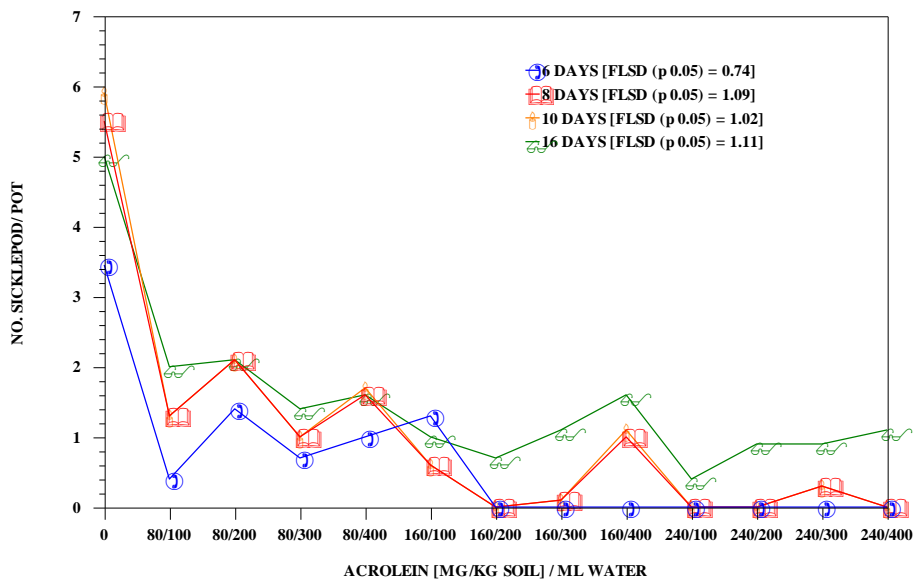


Figure 19E: Number of sicklepod plants per pot in four weed counts (Weed Experiment 9).

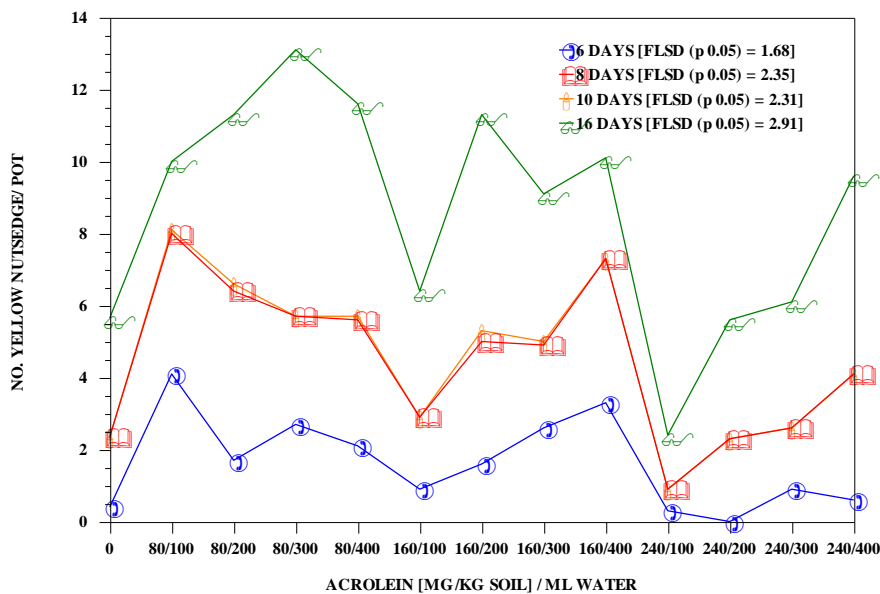


Figure 19F: Number of yellow nutsedge plants per pot in four weed counts (Weed Experiment 9).

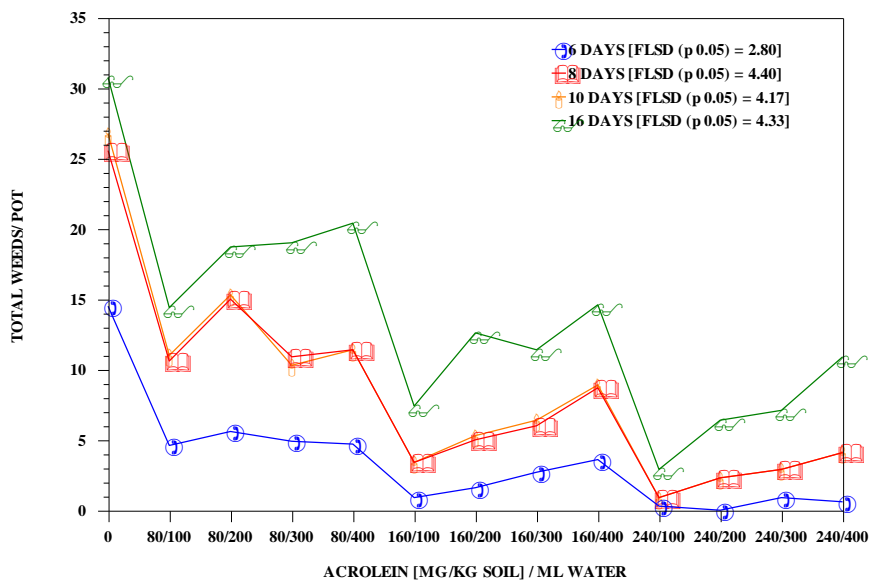


Figure 19G: Number of total weeds per pot in four weed counts (Weed Experiment 9).

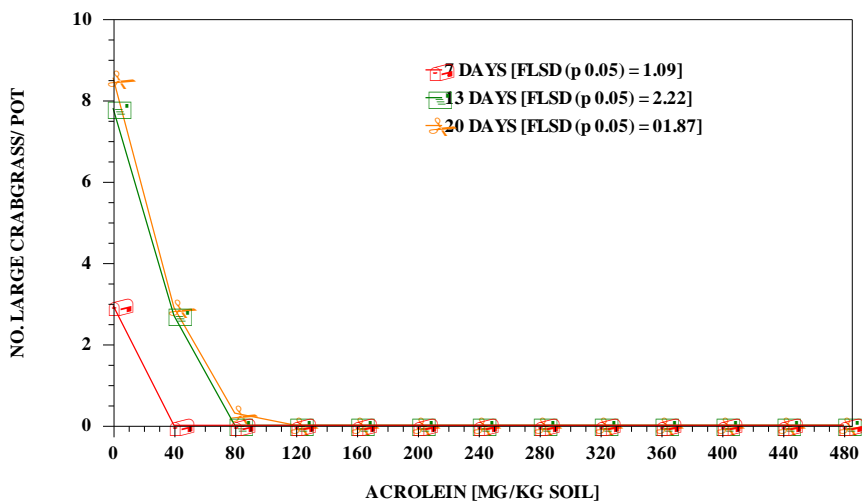


Figure 20A: Number of large crabgrass plants per pot in three weed counts (Weed Experiment 10).

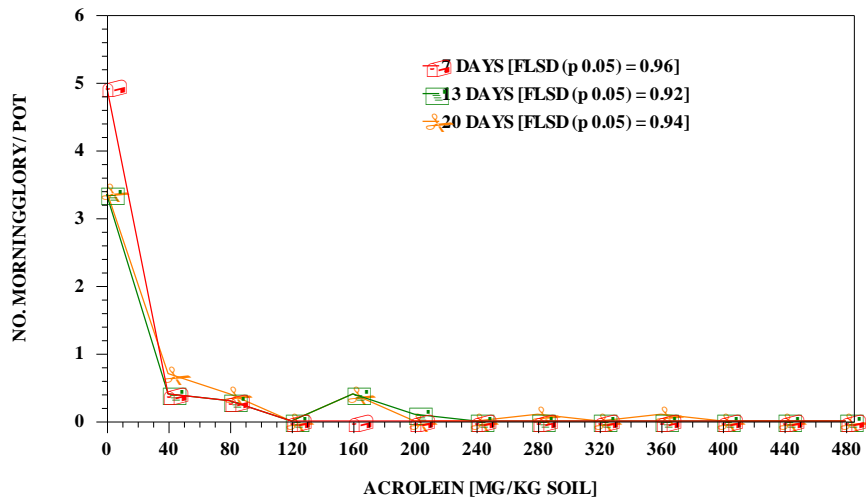


Figure 20B: Number of morningglory plants per pot in three weed counts (Weed Experiment 10).

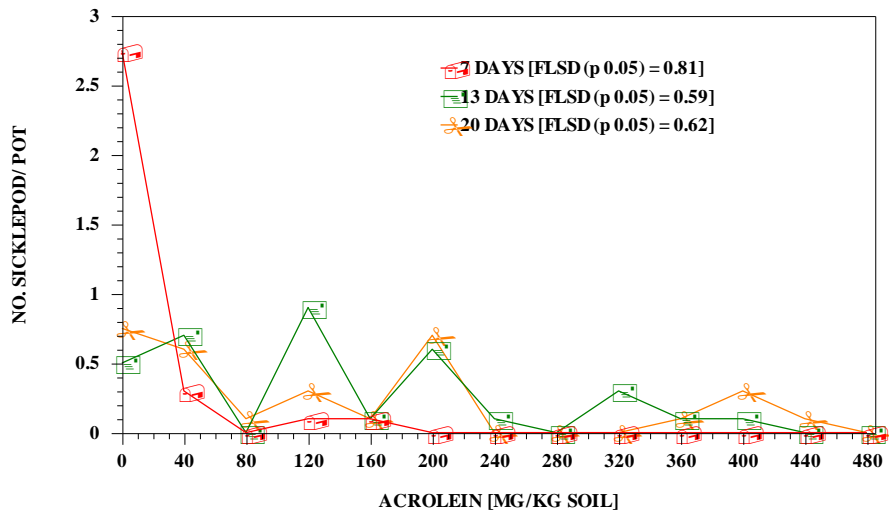


Figure 20C: Number of sicklepod plants per pot in three weed counts (Weed Experiment 10).

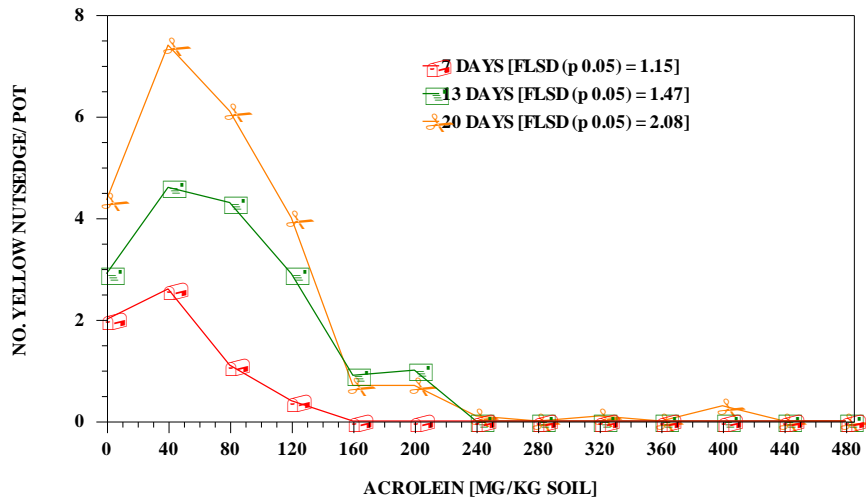


Figure 20D: Number of yellow nutsedge plants per pot in three weed counts (Weed Experiment 10).

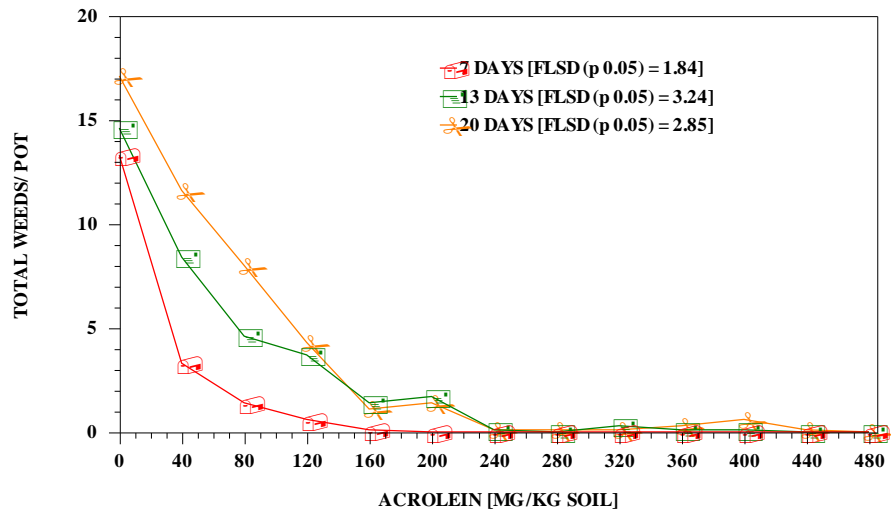


Figure 20E: Total number of weeds per pot in three weed counts (Weed Experiment 10).

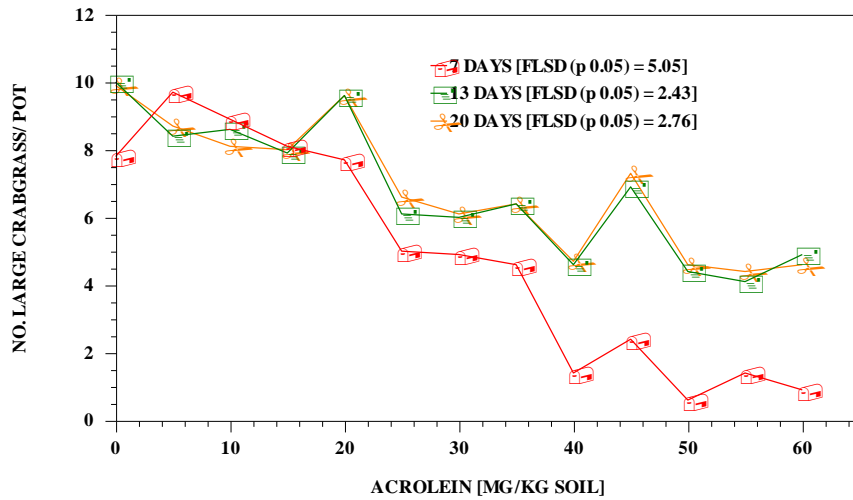


Figure 21A: Number of large crabgrass plants per pot in three weed counts (Weed Experiment 11).

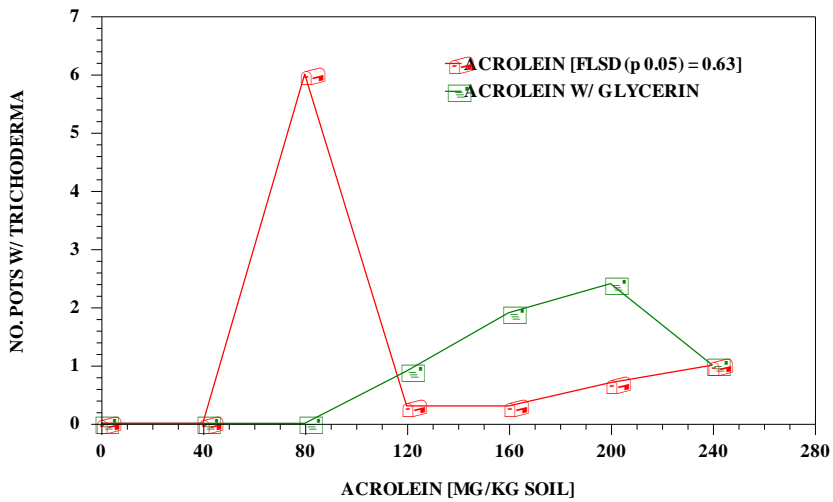


Figure 22A: Rating of *Trichoderma* spp. growing on the soil surface (Weed Experiment 12).

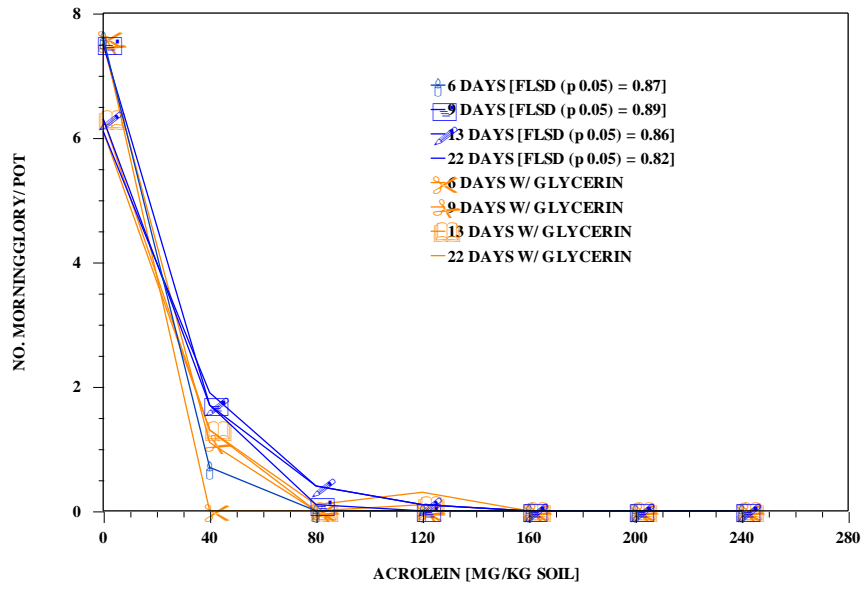


Figure 22B: Number of morningglory plants per pot in four weed counts (Weed Experiment 12).

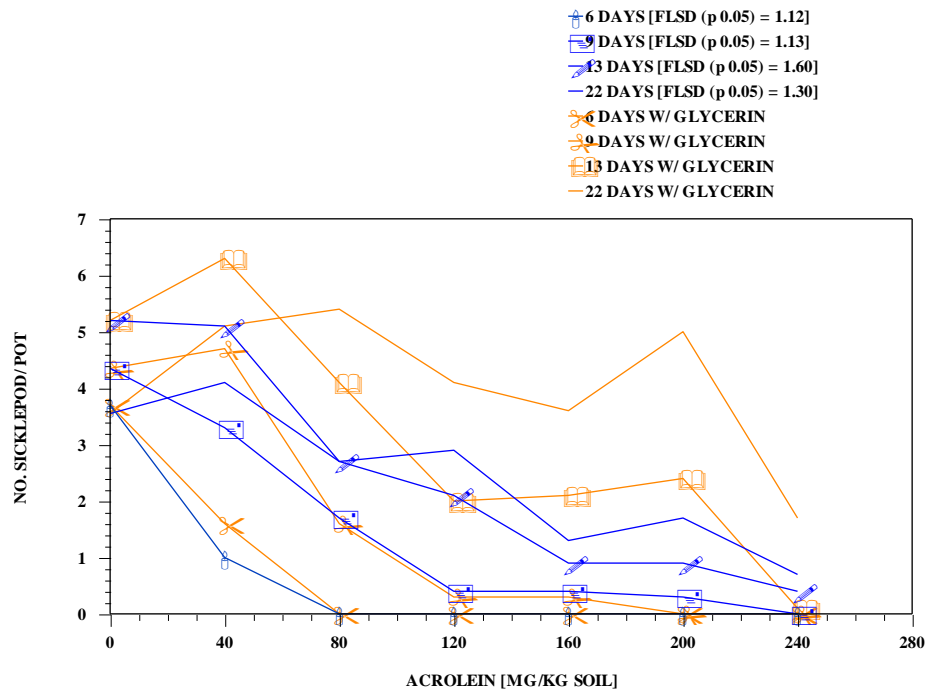


Figure 22C: Number of sicklepod plants per pot in four weed counts (Weed Experiment 12).

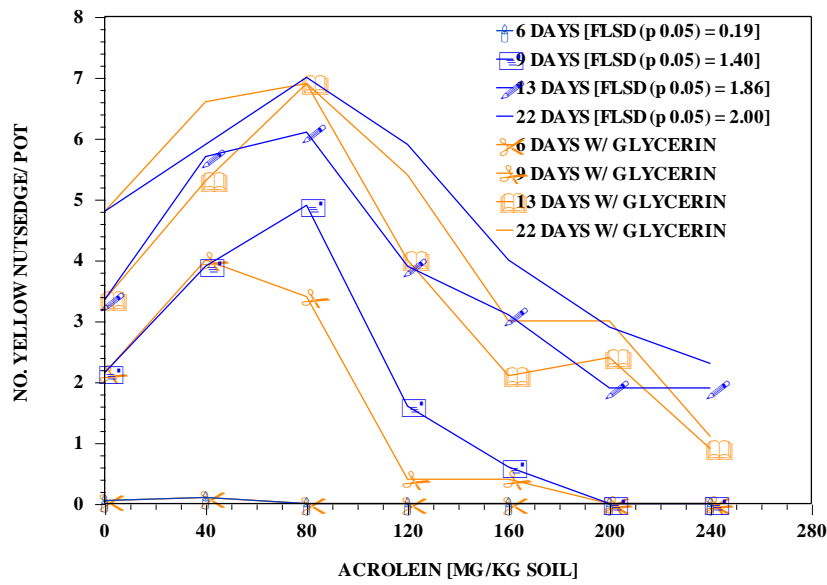


Figure 22D: Number of yellow nutsedge plants per pot in four weed counts (Weed Experiment 12).

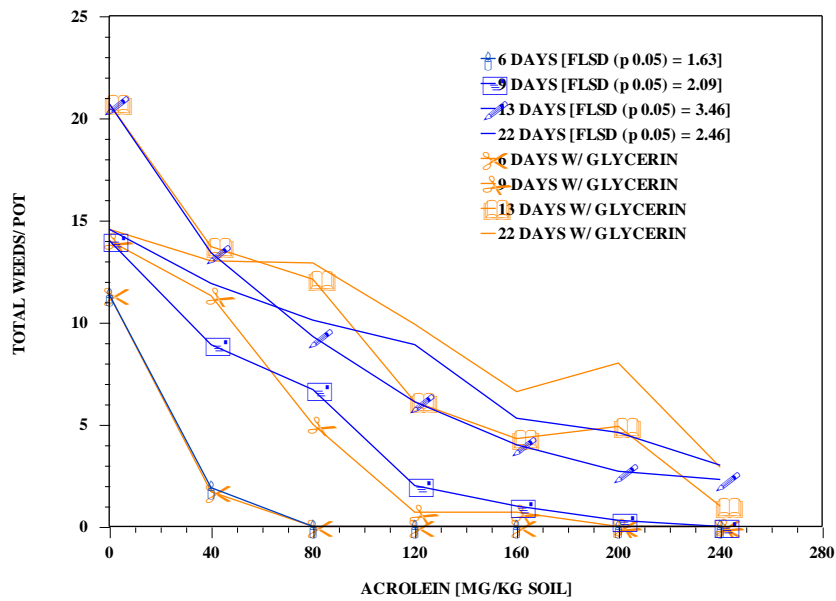


Figure 22E: Total number of weeds per pot in four weed counts (Weed Experiment 12).

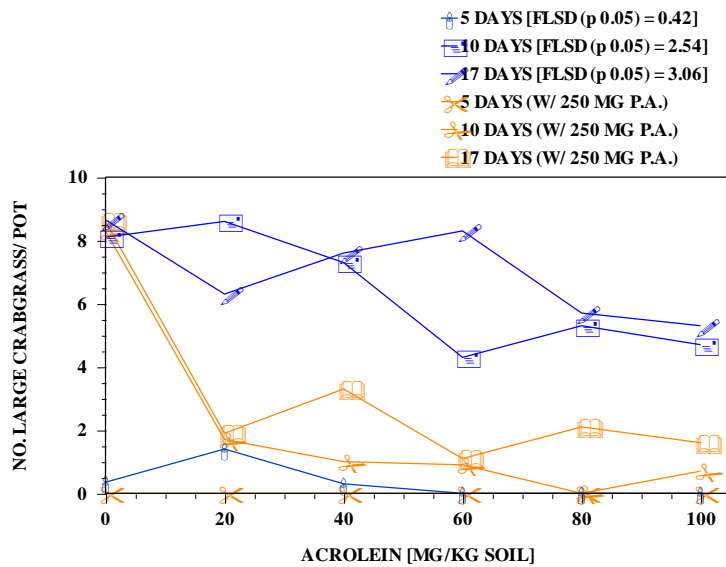


Figure 23A: Number of large crabgrass plants per pot in three weed counts (Weed Experiment 13).

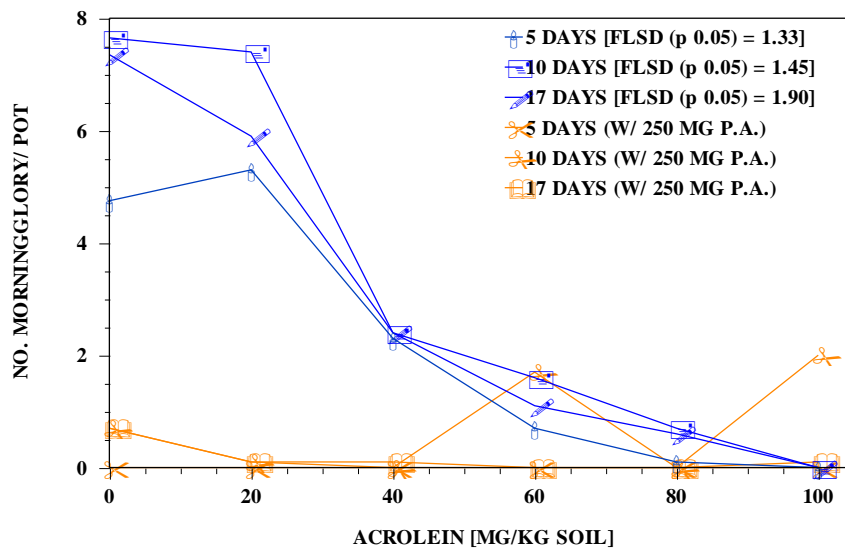


Figure 23B: Number of morningglory plants per pot in three weed counts (Weed Experiment 13).

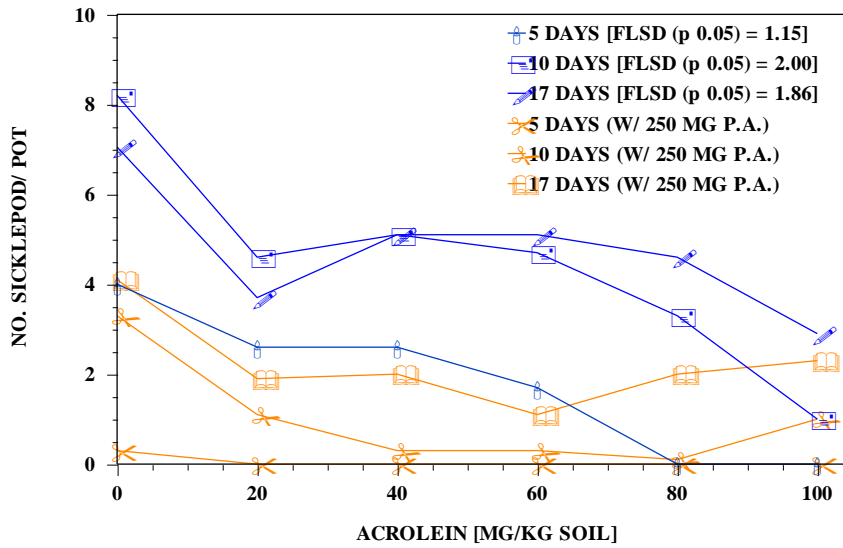


Figure 23C: Number of sicklepod plants per pot in three weed counts (Weed Experiment 13).

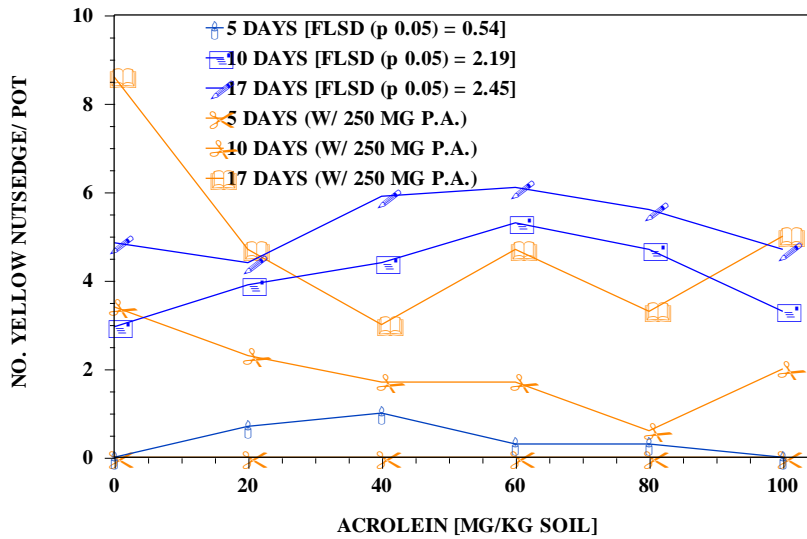


Figure 23D: Number of yellow nutsedge plants per pot in three weed counts (Weed Experiment 13).

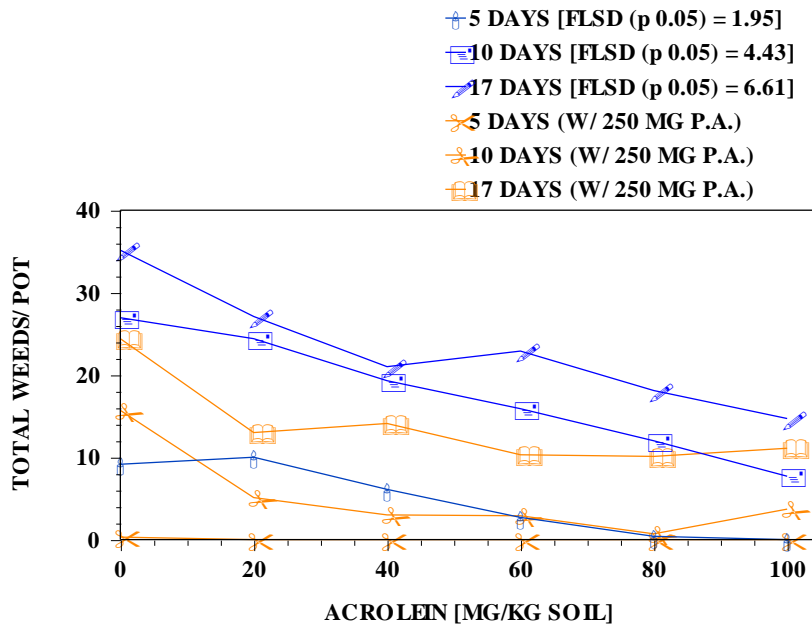


Figure 23E: Total number of weeds per pot in three weed counts (Weed Experiment 13).

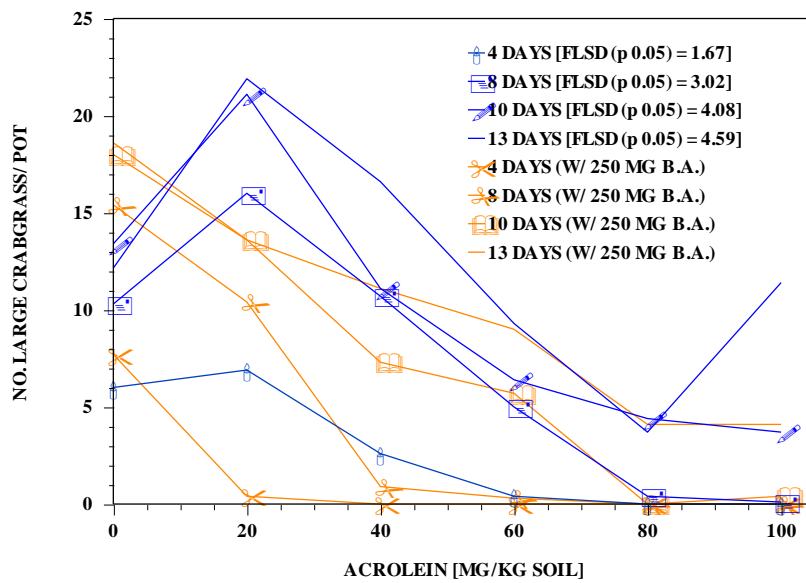


Figure 24A: Number of large crabgrass plants per pot in four weed counts (Weed Experiment 14).

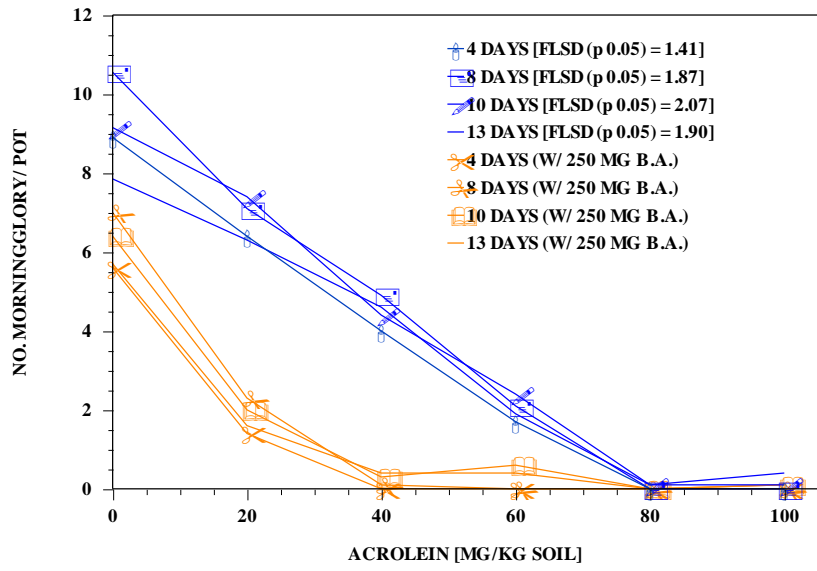


Figure 24B: Number of morningglory plants per pot in four weed counts (Weed Experiment 14).

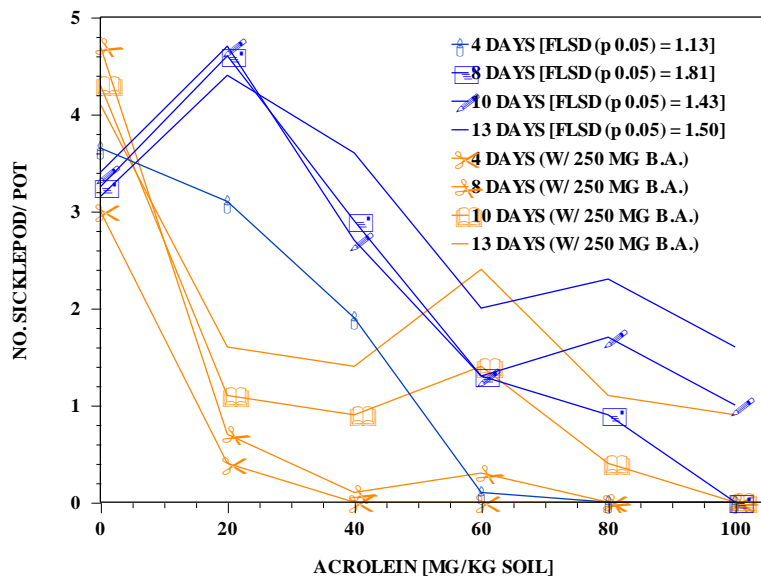


Figure 24C: Number of sicklepod plants per pot in four weeds counts (Weed Experiment 14).

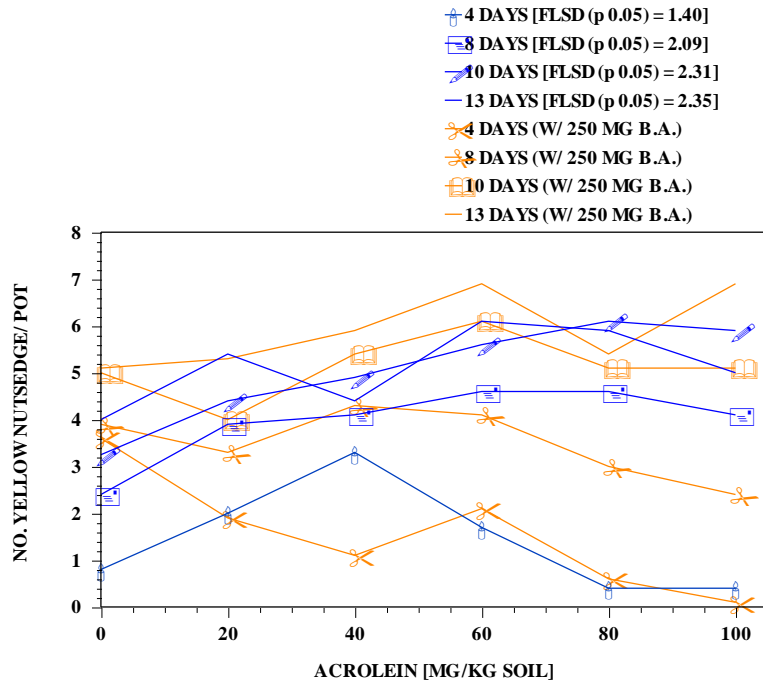


Figure 24D: Number of yellow nutsedge plants per pot in four weed counts (Weed Experiment 14).

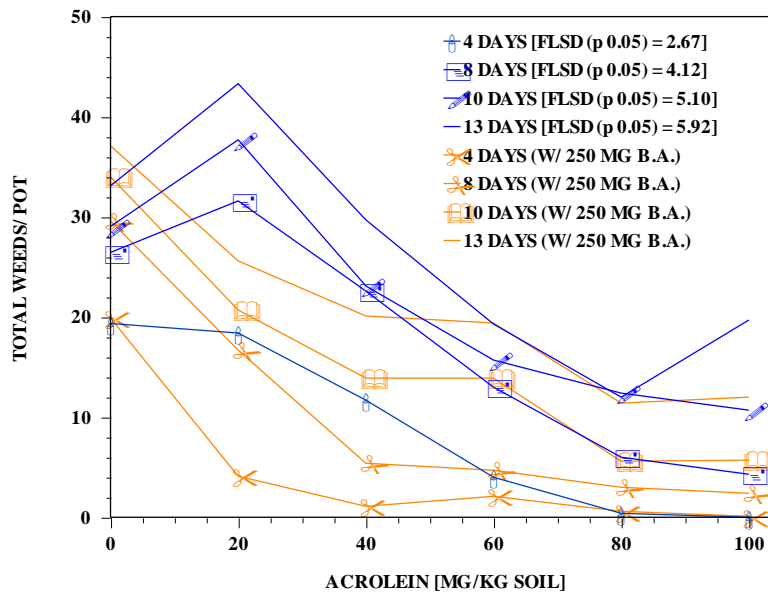


Figure 24E: Total number of weeds per pot in four weeds counts (Weed Experiment 14).

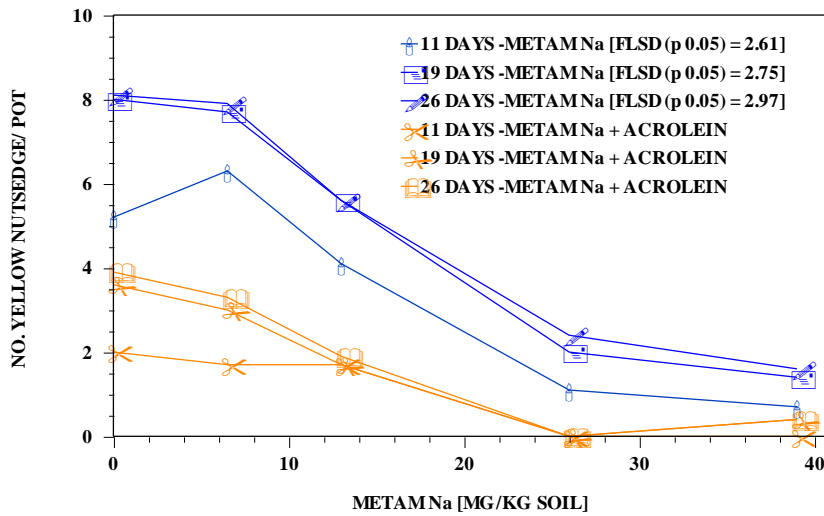


Figure 25A: Number of yellow nutsedge (*Cyperus esculentus*) per pot in three weed counts (Weed Experiment 15).

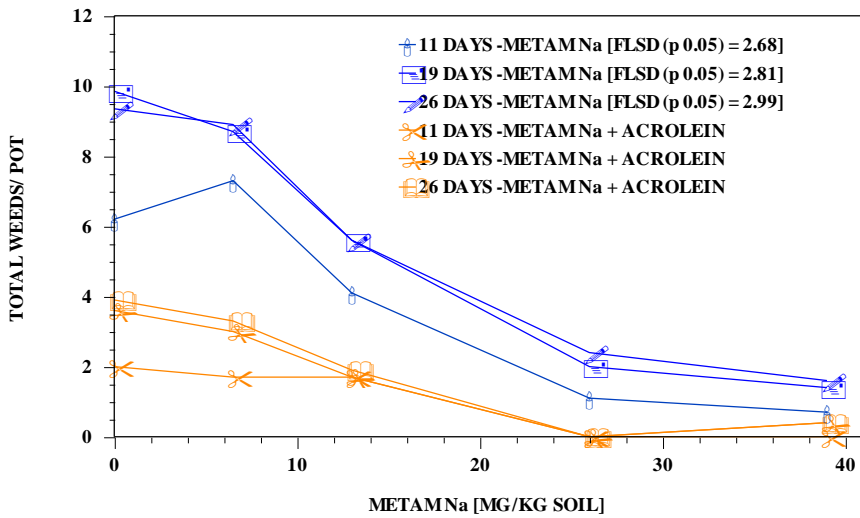


Figure 25B: Total number of weeds per pot in three weed counts (Weed Experiment 15).

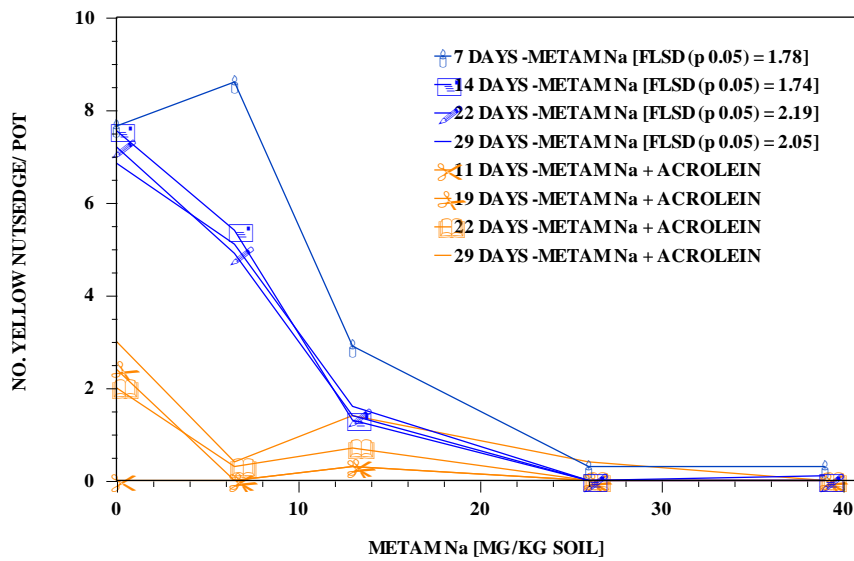


Figure 26A: Number of yellow nutsedge (*Cyperus esculentus*) per pot (Weed Experiment 16).

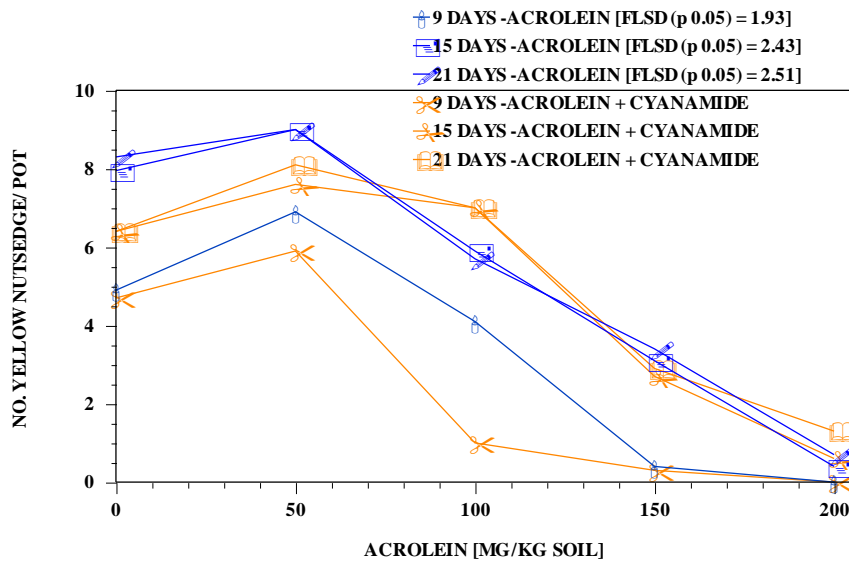


Figure 27A: Number of yellow nutsedge (*Cyperus esculentus*) per pot in three weed counts (Weed Experiment 17).

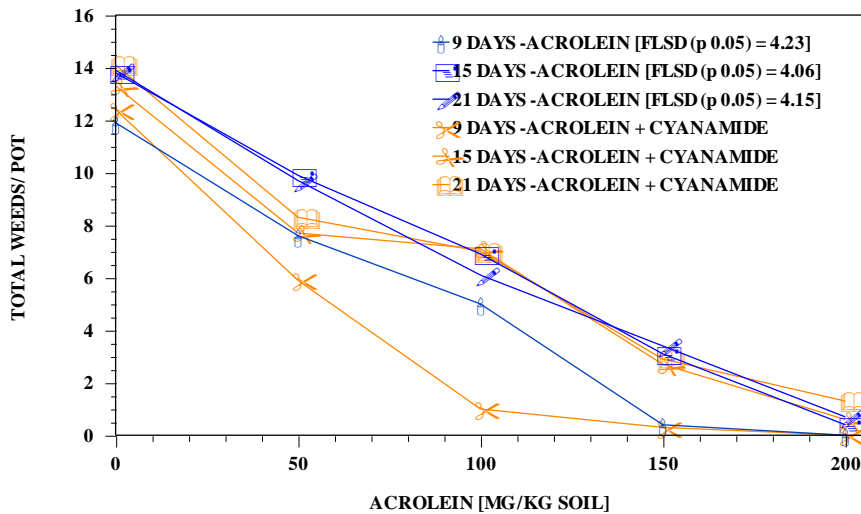


Figure 27B: Total number of weeds counted in three weed counts (Weed Experiment 17).

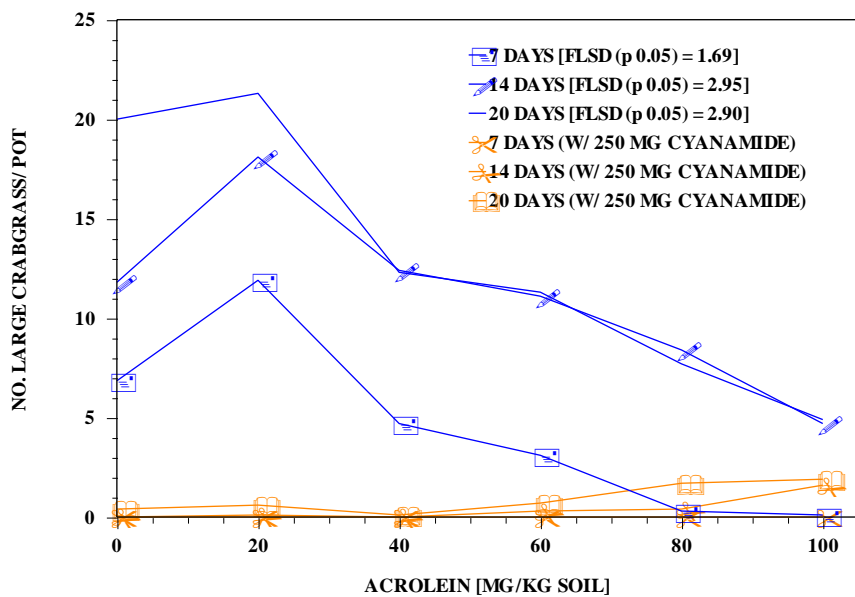


Figure 28A: Number of large crabgrass plants per pot in three weed counts (Weed Experiment 18).

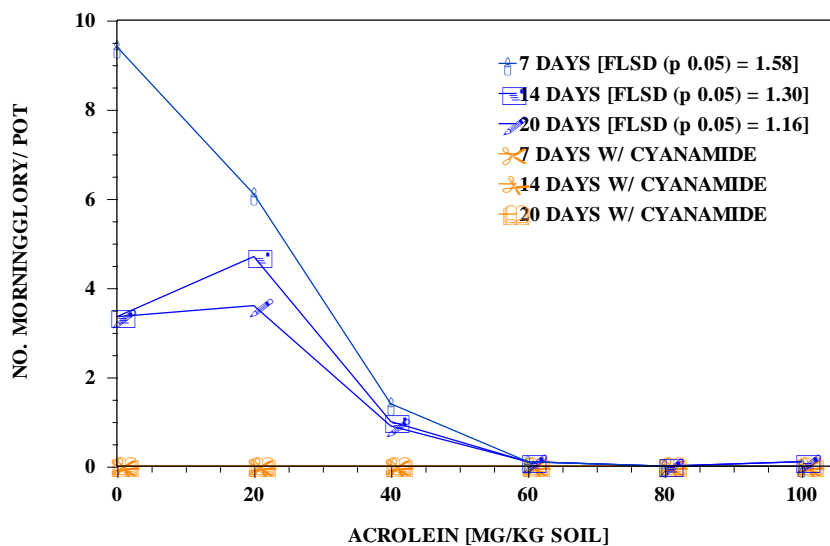


Figure 28B: Number of morningglory plants per pot in three weed counts (Weed Experiment 18).

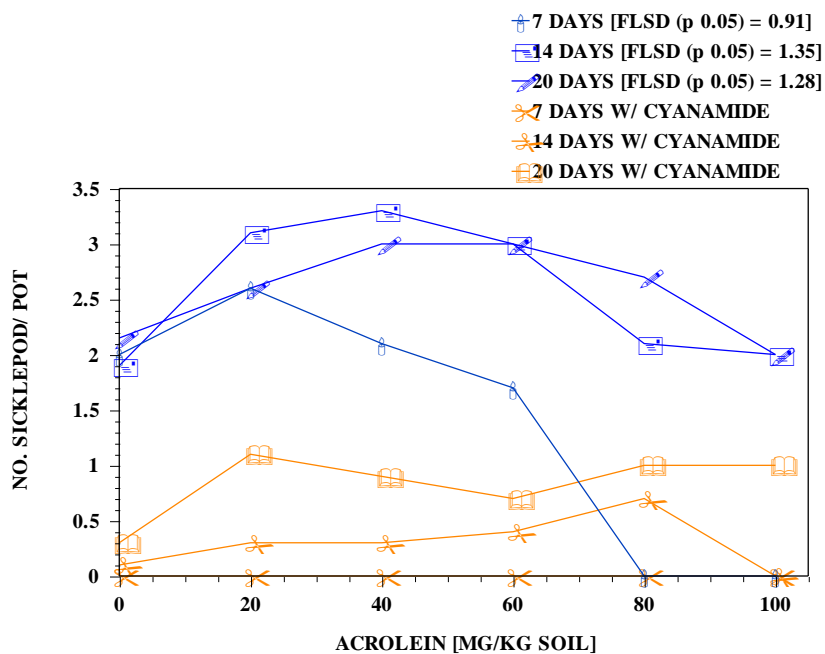


Figure 28C: Number of sicklepod plants per pot in three weed counts (Weed Experiment 18).

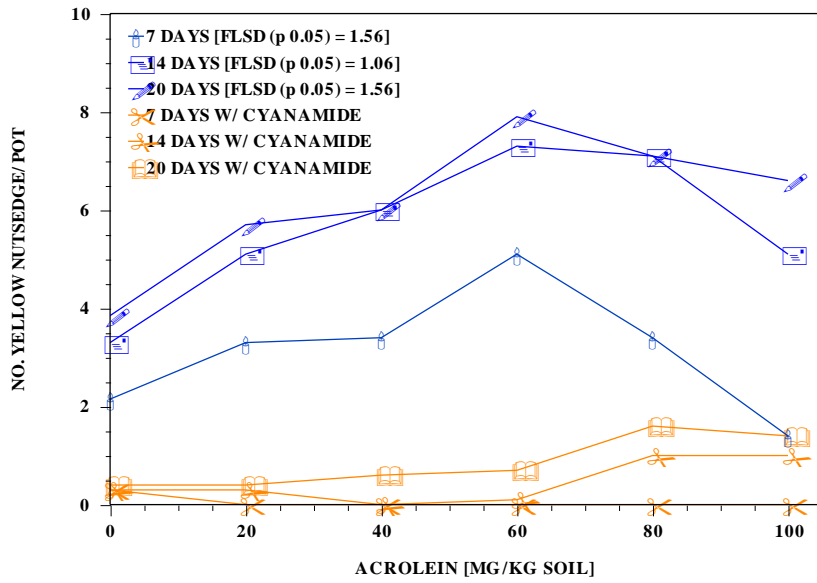


Figure 28D: Number of yellow nutsedge plants per pot in three weed counts (Weed Experiment 18).

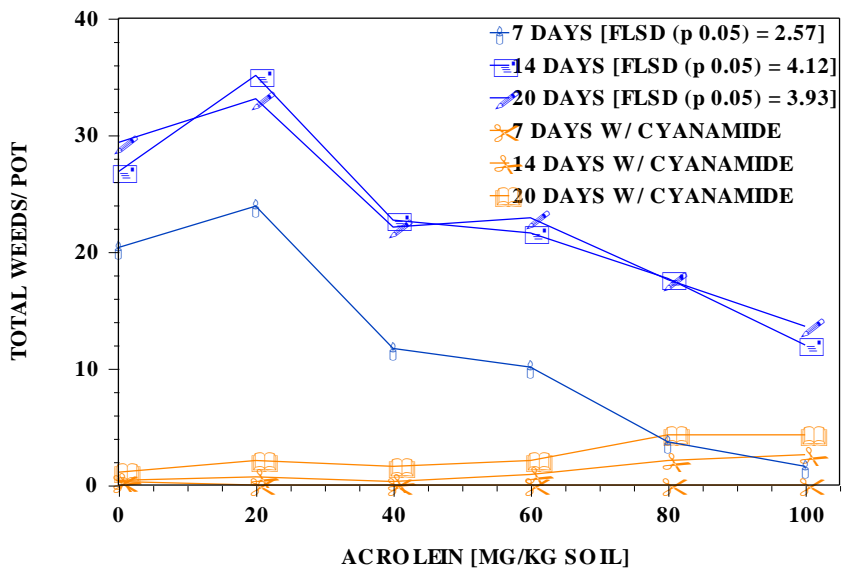


Figure 28E: Total number of weeds per pot in three weed counts (Weed Experiment 18).

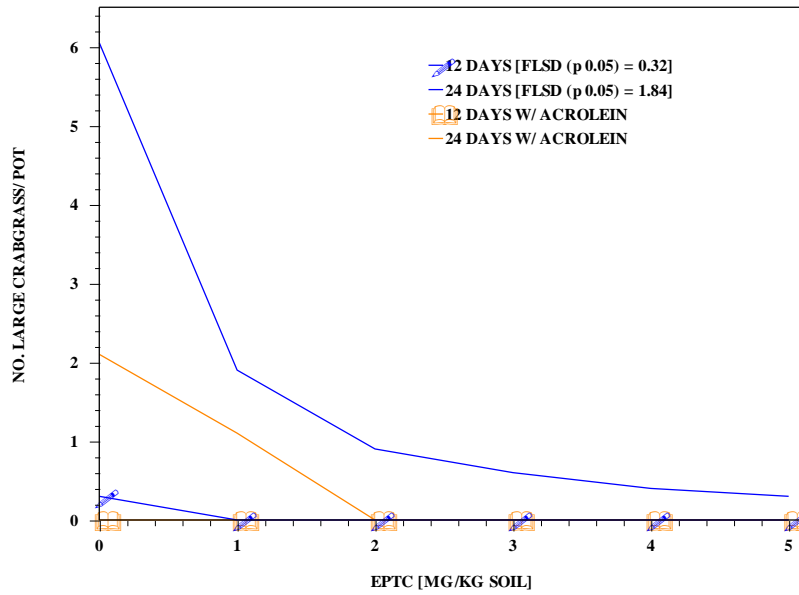


Figure 29A: Number of large crabgrass plants per pot in the last two weed counts (Weed Experiment 19).

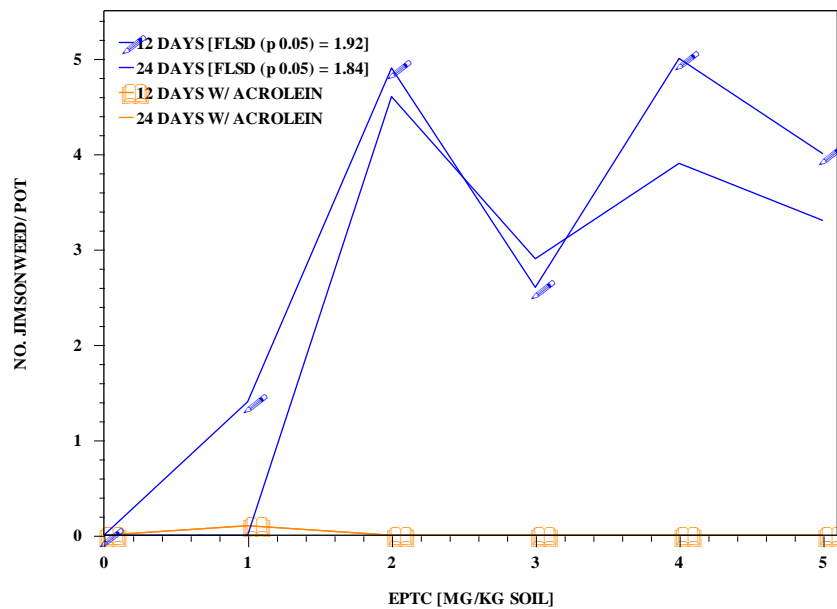


Figure 29B: Number of jimsonweed plants per pot in the last two weed counts (Weed Experiment 19).

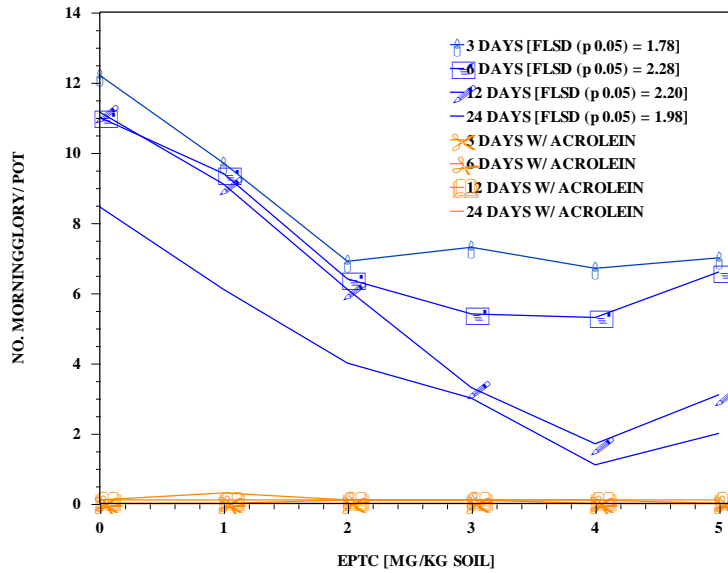


Figure 29C: Number of morningglory plants per pot in four weed counts (Weed Experiment 19).

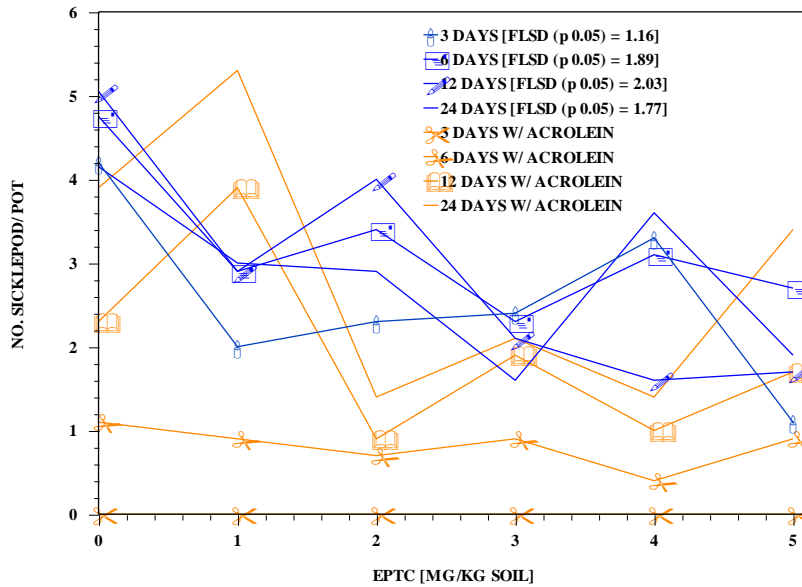


Figure 29D: Number of sicklepod plants per pot in four weed counts (Weed Experiment 19).

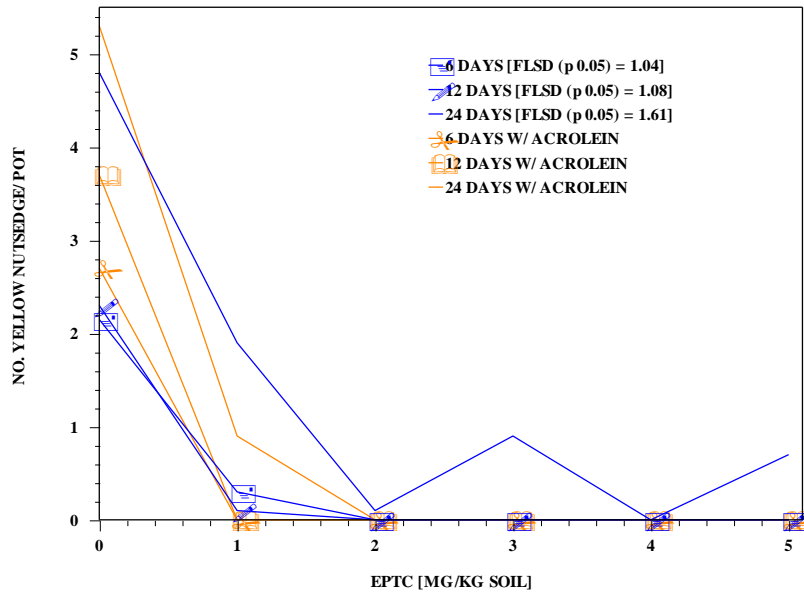


Figure 29E: Number of yellow nutsedge plants per pot in three weed counts (Weed Experiment 19).

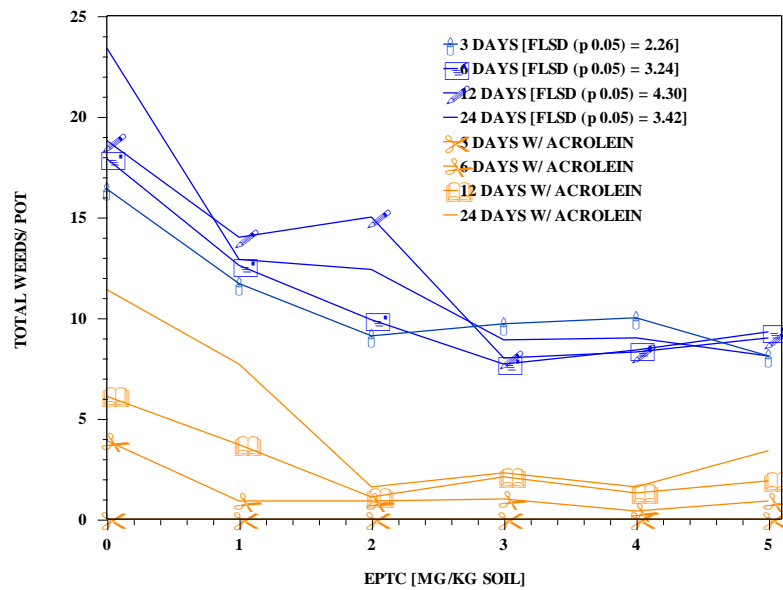


Figure 29F: Total number of weeds per pot in four weed counts (Weed Experiment 19).

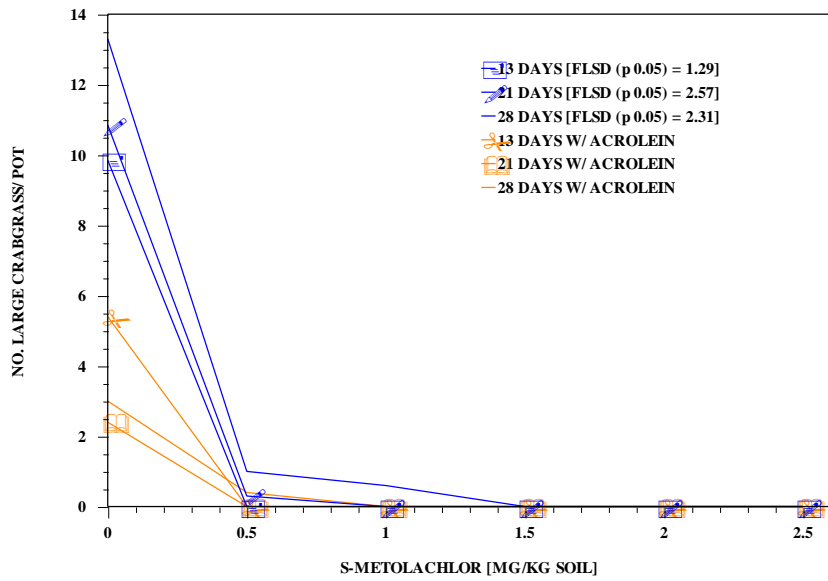


Figure 30A: Number of large crabgrass plants per pot in three weed counts (Weed Experiment 20).

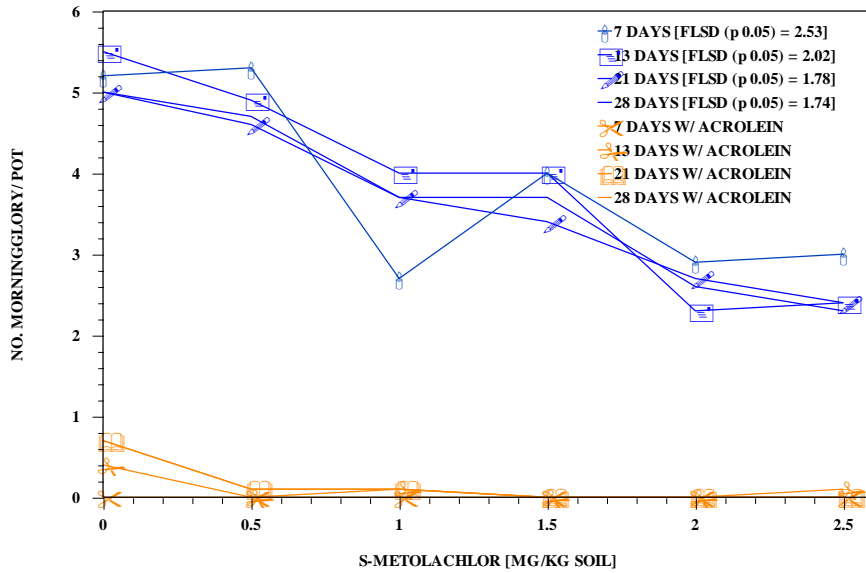


Figure 30B: Number of morningglory plants per pot in four weed counts (Weed Experiment 20).

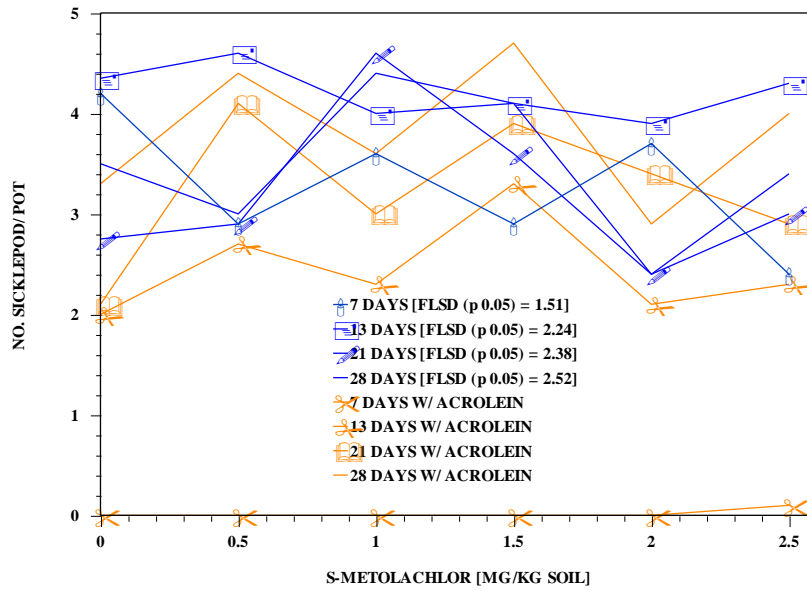


Figure 30C: Number of sicklepod plants per pot in four weed counts (Weed Experiment 20).

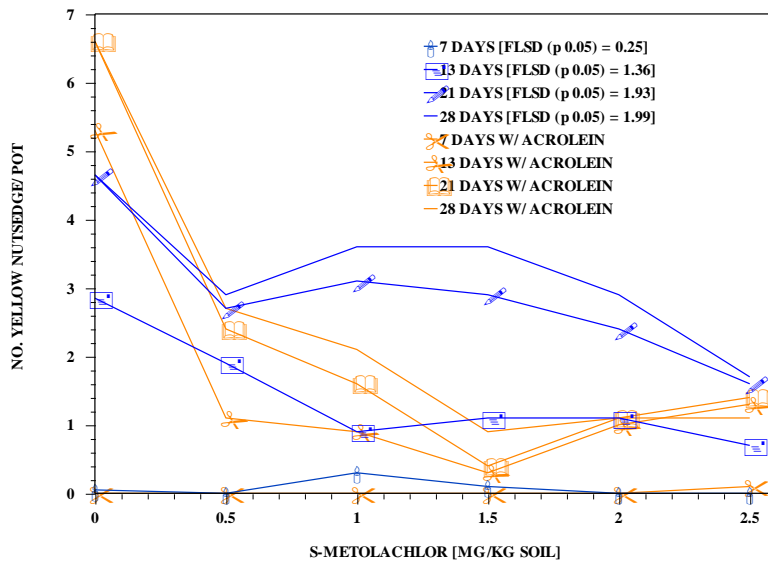


Figure 30D: Number of yellow nutsedge plants per pot in four weed counts (Weed Experiment 20).

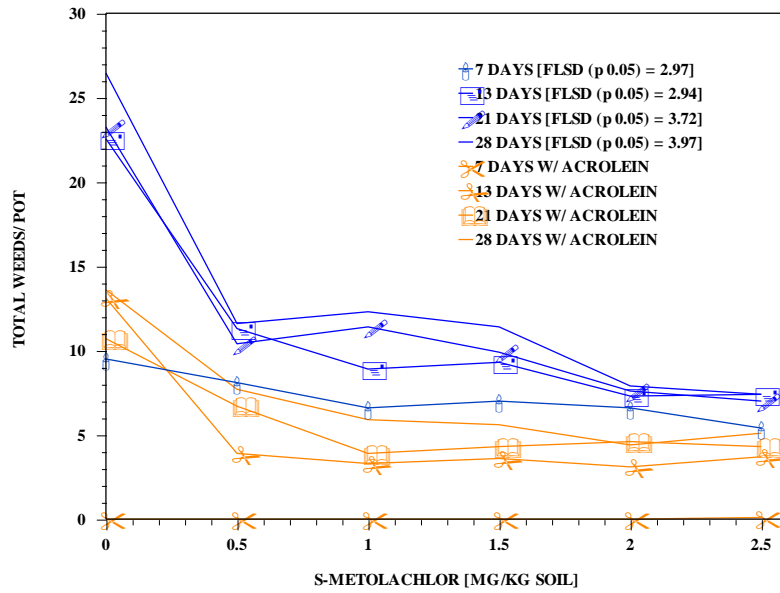


Figure 30E: Total number of weeds per pot in four weed counts (Weed Experiment 20).

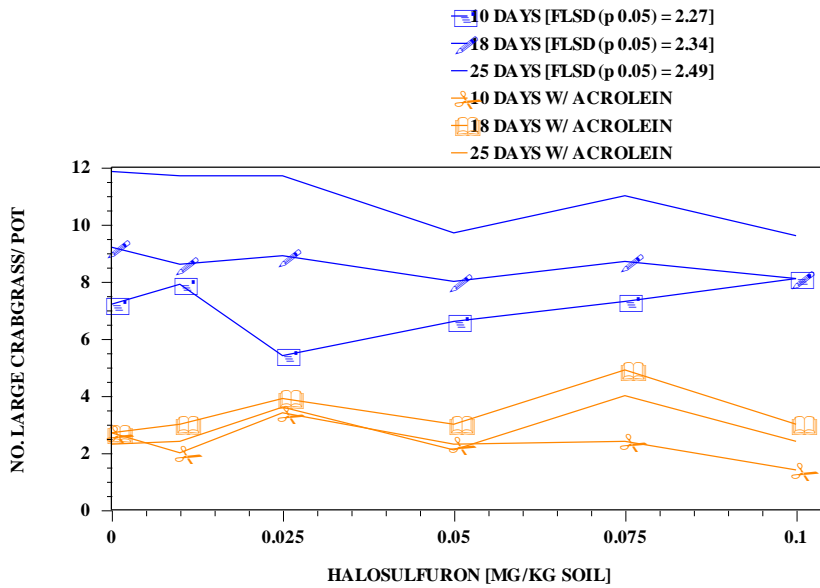


Figure 31A: Number of large crabgrass plants per pot in three weed counts (Weed Experiment 21).

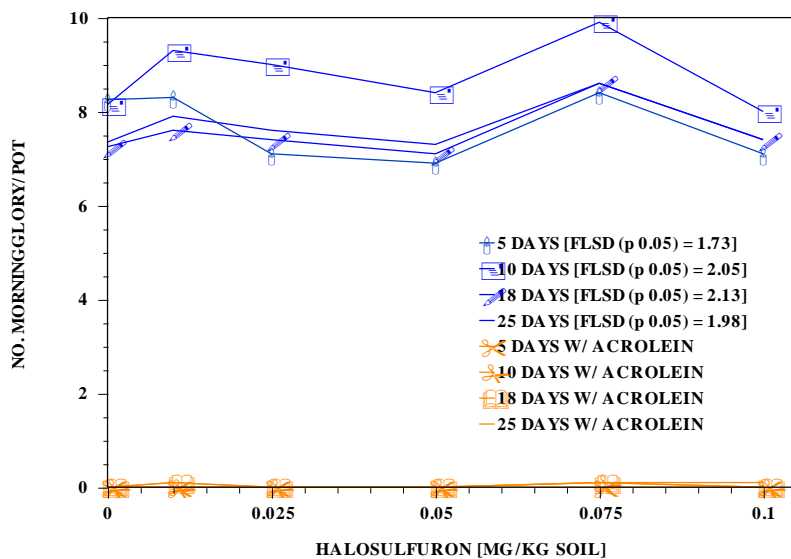


Figure 31B: Number of morningglory plants per pot in four weed counts (Weed Experiment 21).

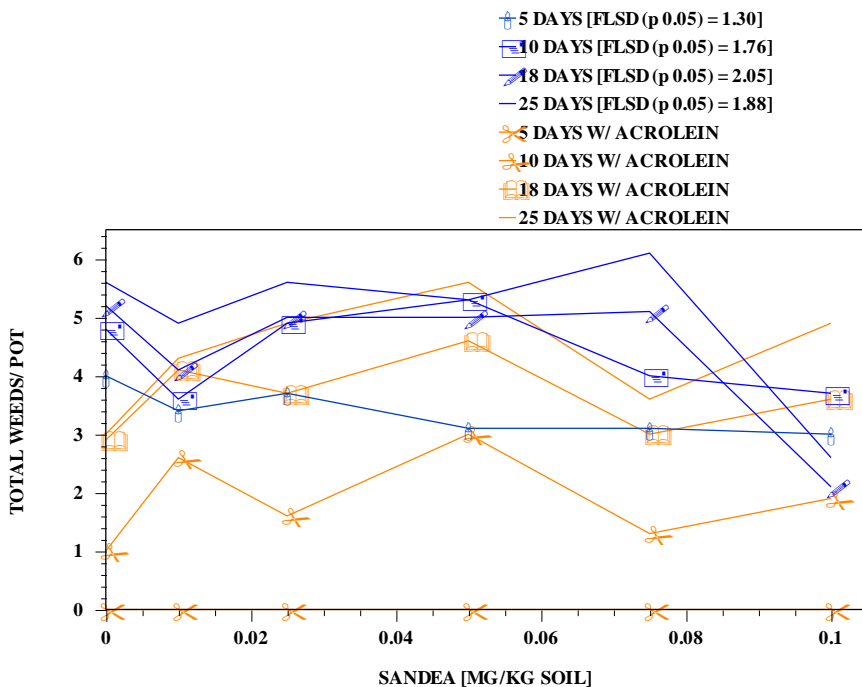


Figure 31C: Number of sicklepod plants per pot in four weed counts (Weed Experiment 21).

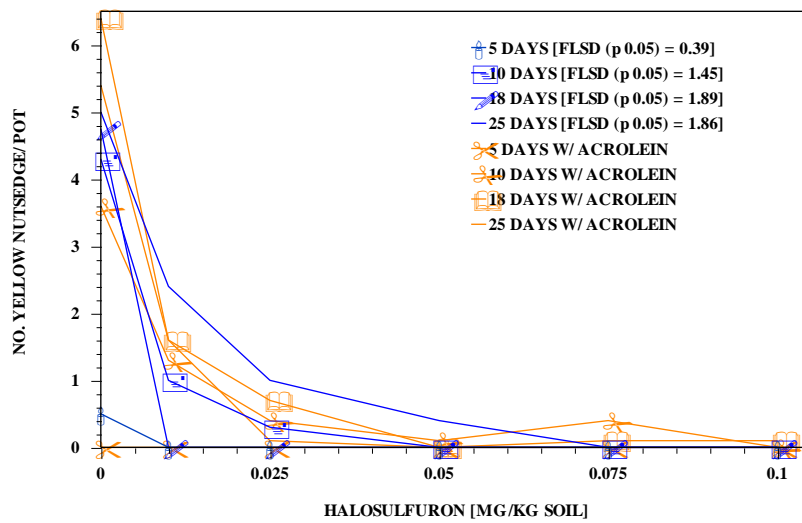


Figure 31D: Number of yellow nutsedge plants per pot in four weed counts (Weed Experiment 21).

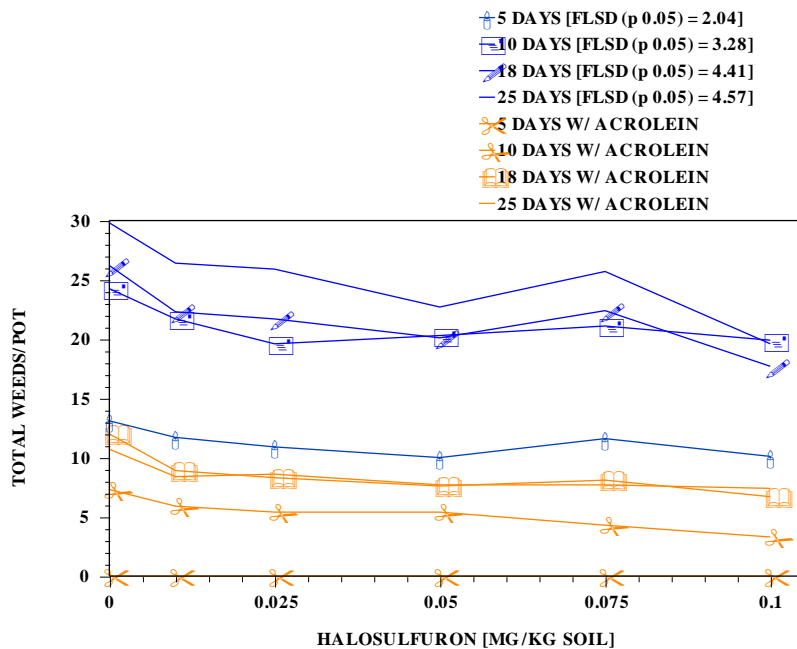
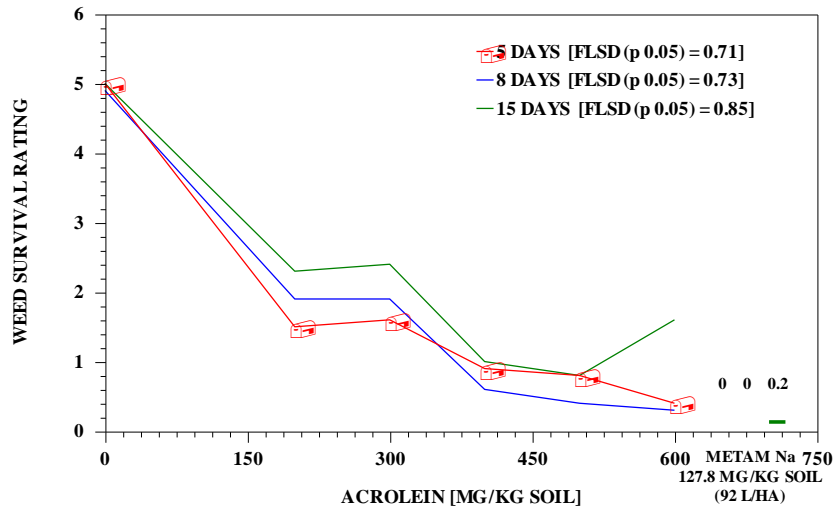
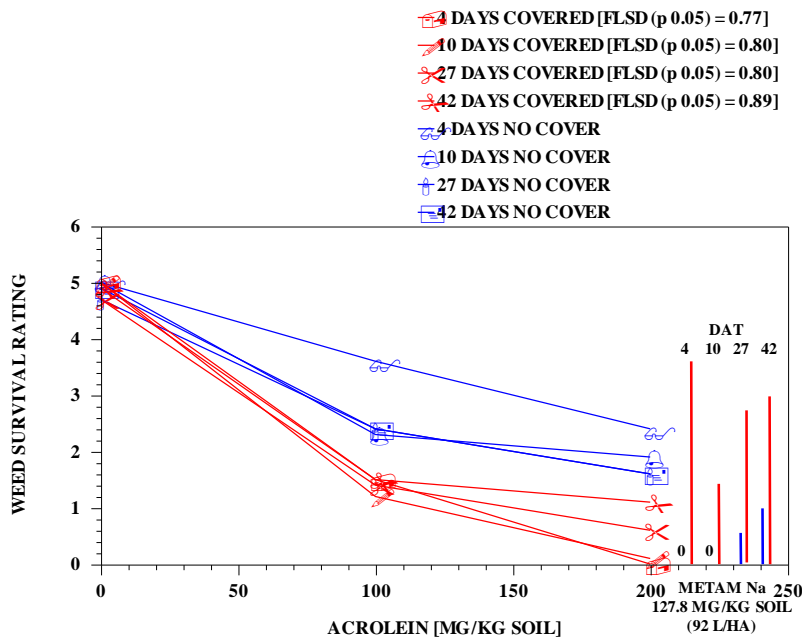


Figure 31E: Total number of weeds per pot in four weed counts (Weed Experiment 21).



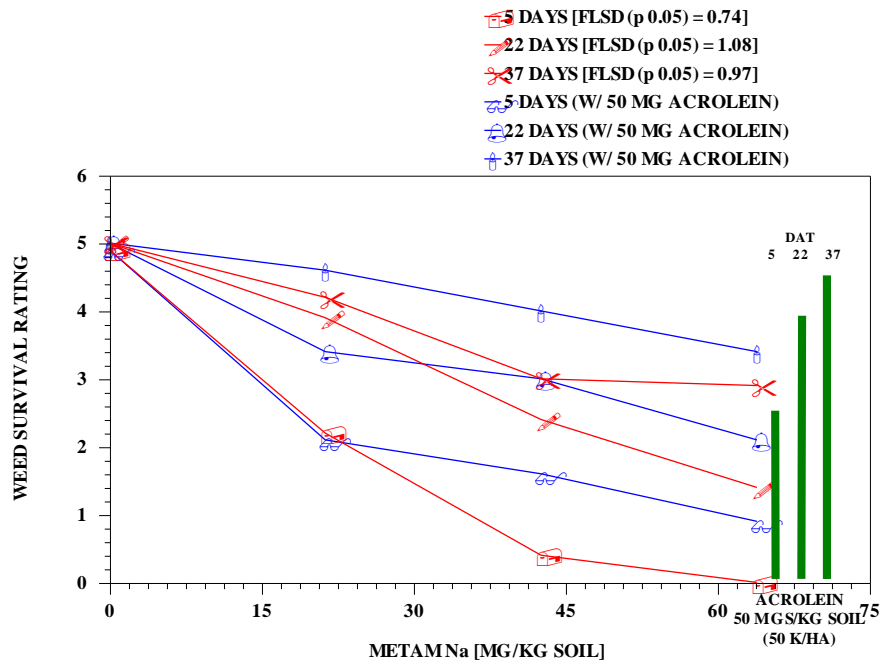
WEED RATING SCALE: 5= NO WEED CONTROL, 0= COMPLETE WEED CONTROL

Figure 32A: Microplot I weed ratings at 5, 8, and 15 days after treatment (Weed Experiment 22).



WEED RATING SCALE: 5= NO WEED CONTROL, 0= COMPLETE WEED CONTROL

Figure 33A: Microplot weeds ratings of covered and non-covered plots for four weed ratings (Weed Experiment 23).



WEED RATING SCALE: 5= NO WEED CONTROL, 0= COMPLETE WEED CONTROL

Figure 34A: Microplot weed survival rating for metam sodium and acrolein treatments for three weed ratings (Weed Experiment 24).

IV. EFFECTS OF ACROLEIN ON SOIL MICROBIOLOGY AND SOIL ENZYMATIC ACTIVITIES

A. ABSTRACT

In continuing efforts to develop alternatives to methyl-bromide, -enal compounds with pesticidal activities similar to those of methyl-bromide were tested for efficacy against agricultural pests. Several compounds from this group have potential as pesticides, but acrolein is the only one registered for use in the U.S.A. Acrolein is currently labeled as an aquatic herbicide. Recently, reports of nematicidal activity and agricultural use to control weeds have been published suggesting that acrolein may be used as an alternative to methyl bromide. All reports to date have focused on the efficacy of the compound on agricultural pests and no work has been done to elucidate the effects of acrolein on the soil microflora. When considering chemical alternatives to methyl bromide it is essential that the effects of the alternative compound on the soil microflora be considered. Dangerous soil microbiological voids and selection for plant pathogenic micro-organisms is a common problem often associated with broad-spectrum, biocidal compounds like methyl bromide.

Tests were conducted to determine the effects of soil-applied acrolein on the microflora and several key biochemical processes of the soil. Soil microbial survey included soil plating using media selective for fungi, bacteria, and actinomycetes for general identification and enumeration. Soil enzymatic activities measured included:

catalase, α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, chitinase, phosphatase, sulfatase, and urease. The effects of acrolein on soil pH and electrical conductivity were also determined

Soil Biochemistry- As acrolein rates increased, most of the enzymatic activities measured decreased. This is indicative of biocidal activity. With the exception of sulfatase, enzymatic activities revealed in the final soil samples remained less than those in the controls and the activities tended to decrease as rates of acrolein increased. Acrolein resulted in minimal changes of soil pH and electrical conductivity.

Soil Microbiology- Bacterial colonies decreased as rates of acrolein increased to 200 mg/kg soil. In contrast, the number of actinomycetes and fungi increased as rates of acrolein increased. This suggests that acrolein is not a biocide; rather that acrolein is a selective pesticide when applied to the soil. Furthermore, while the number of fungal colonies increased, diversity decreased to nearly consist of one fungal genus:

Trichoderma. This genus is known for species with antagonistic effects on plant pathogens. The *Trichoderma* data from the soil plating follow a very similar trend as the data taken on visual rating of *Trichoderma* presence on the surface of the soil following acrolein treatments. As the rate of acrolein applied to the soil increased, morningglory germination and survival increased indicating that acrolein may reduce seedling diseases.

Unlike typical biocides, acrolein exhibited properties of a selective pesticide when applied to the soil. Acrolein stimulated *Trichoderma* spp., a desirable group of micro-organisms. These activities coincided with improved plant growth and nematode control. Results suggest that acrolein may be used to control pests and improve plant growth without creating dangerous microbiological voids in the soil.

B. INTRODUCTION

While acrolein is found readily in nature, it was discovered by man over 100 years ago. Since its discovery, a variety of uses have been developed, primarily in the fields of polymer and plastic chemistries. For over 50 years acrolein has also been used to control a variety of aquatic weeds, algae, and other aquatic pests such as snails and mussels. It has been used to clear biological blockage from water-cooling-systems and even to clear bacterial blockage from large oil pipe-lines (Donohue et al., 1966; Rijstenbil et al., 1981). While several patents claim that acrolein can be used to disinfest soils of plant pathogenic fungi, bacteria, and nematodes, no studies were found that detailed specific rates and methods of application required for effective control of these deleterious organisms as (Kreutzer, 1962; Krenzer, 1971; Racusen and Legator, 1962; Roe *et al.*, 1996; Bockowski and Davis, 1999; Bockowski and Davis, 2001; and Allan and Schiller, 2007).

Due to an international agreement, the U.S. will stop using methyl bromide, an ozone depleting compound, to disinfest agricultural soils prior to planting. With the impending loss of this compound, many agricultural researchers are exploring new methods and new compounds that can be used as alternatives to methyl bromide. Acrolein is one compound that has been recently considered for use as an alternative to methyl bromide in agriculture. Recent reports, including the data provided in this dissertation, show potential for this compound to be used as an effective alternative to methyl bromide for the control of plant pathogenic nematodes and weeds (Rodríguez-Kábana et al., 2003).

A problem often associated with methyl bromide and other broad-spectrum biocides is the formation of a microbiological void in the soil after treatment. This void can allow for plant pathogenic organisms to flourish in the soil with no other organisms to compete with for nutrients and colonization. This study was conducted to determine what effects acrolein has on pathogenic soil microorganisms and the effects of the chemical on the soil microflora.

The study consisted of greenhouse and laboratory experiments. The greenhouse experiments addressed: (a) dosimetry and methods to disinfest plant bulbs prior to planting, (b) the efficacy of acrolein for control of soil-borne fungal seedling diseases, and (c) the effects of the chemical on the soil microflora and on key biochemical activities of the soil.

C. EXPERIMENTS

Caladium Bulb Experiments: Caladiums are commonly sold as dormant bulbs that are often infested with fungal pathogens. These pathogens can either diminish the quality of the caladiums or possibly kill them. Two experiments were conducted to investigate the potential for acrolein to be used to disinfest caladium bulbs prior to planting.

Microbiology Experiment 1: An experiment was conducted to test the efficacy of acrolein to disinfest caladium bulbs by dipping the bulbs in an acrolein solution for several durations of time.

Materials and Methods:

Seven caladium bulbs (*Caladium bicolor*, bulb size No. 3) were selected from a diseased lot at random and placed in a 25 cm diameter sieve. The sieve and bulbs were placed in a pan with 0.1% acrolein solution for 0, 1, 2, 4, 6, 8, and 10 minutes to disinfest the surface of the bulbs. After remaining in the acrolein solution for the requisite amount of time, the sieve with bulbs was removed, the sieve was shaken to remove any excess solution, and the bulbs were planted in pots (1/2 the height of the previously described standard PVC pot) containing 0.5 kg of a 2:1 sand: peat moss mixture. Pots were arranged on a greenhouse bench in a randomized complete block design with seven replications per treatment and then watered. Data were collected on the number of plants per pot and number of leaves per pot at 46, 74, 89, and 98 days after treatment. The experiment was terminated 98 days after treatment and data were collected on the total fresh weight of the plants (weight of fresh shoots and weight of fresh roots).

Data Analyses:

Data were analyzed using SAS software; Fisher's least significant differences ($P=0.05$) were calculated when F values were significant. Unless otherwise noted, statements made in the results of this experiment and all others in this chapter are for significant differences at $P=0.05$ level of probability.

Results:

Bulbs dipped in acrolein for 4 minutes had more plants per pot than any other treatment (Fig. 35A). Although no other treatments differed statistically from the controls, bulbs dipped in acrolein for six minutes or longer tended to have fewer plants per pot than the controls (Fig. 35A). The number of leaves per pot followed the same

trends as the number of plants per pot; bulbs dipped for four minutes having significantly more leaves than all other treatments and those dipped for six minutes or more tending to have fewer leaves than the controls (Fig. 35B). Bulbs treated in acrolein for one to six minutes resulted in plants that weighed more than those in the controls (Fig. 35C). When treated six minutes or longer, the total fresh weight of the plants begins to decline (Fig. 35C).

Conclusions:

Dipping caladium bulbs in an acrolein solution for 2 or 4 minutes improved caladium plants. Dipping the bulbs in the solution for ≥ 6 minutes did not improve caladium plants and may have caused a decline in plant growth.

Microbiology Experiment 2: A second experiment was setup to test the efficacy of acrolein at disinfesting caladium bulbs prior to planting. Duration of dipping was fixed at one and two minutes and concentrations of acrolein were varied.

Materials and Methods:

Red caladium, cultivar 'Buck', was used in this experiment. Dipping was performed as described in Microbiology Experiment 1. Seven bulbs were dipped for each treatment of 0.05, 0.1, 0.2, 0.4, 0.6, and 0.8 % acrolein solutions for one minute, and seven bulbs were dipped in each of the same acrolein solutions for two minutes. Bulbs were planted and pots were arranged as described in Microbiology Experiment 1. The number of plants per pot was determined at 36, 40, 48, 60, 69, 76, 97, and 111 days after treatment. At the termination of the experiment, the number of leaves were counted, total fresh plant weight in grams was recorded, a root condition rating was taken (rating scale

1 - 5; 1= best, 5= worst), and a foliar visual appearance rating was (rating scale 1 - 5; 1= best, 5= worst).

Results:

The data did not show any clear differences among treatments. The number of plants per pot tended to be stimulated with the 0.05% acrolein/one-minute treatment, while the numbers of plants tended to decrease with the 0.1% acrolein/two-minute treatment (Figs. 36A and 36B). Increasing rates above 0.1% acrolein did not increase the number of plants or leaves per pot, nor did it improve foliar or root ratings (Figs. 36A, 36B, 36C, and 36D).

Conclusions:

The 0.05 - 0.1% range of acrolein is best for disinfesting caladium bulbs; there were no improvements with higher concentrations. The shorter dipping duration may be best.

Drench Applications and Seedling Diseases:

Three bioassay experiments were designed to determine the relationship between drench-applied acrolein treatments and soil-borne seedling diseases. Seedling diseases attack plant while they are most susceptible and can reduce yields or prohibit production in some areas.

Microbiology Experiment 3:

This experiment studied the effects of drench-applied acrolein on soil infested with cotton seedling diseases.

Materials and Methods:

Soil for the experiment was from a Tallassee, AL cotton-field with severe seedling disease problems. Pots (previously described) were filled with one kg aliquots of this soil and placed on a greenhouse bench. Acrolein was delivered in 100 ml per pot final volume drenches at rates: 10, 20, 30, 40, 50, and 60 mg/kg soil prepared from a 1% solution. Two water-treated controls were included. After treatment, pots were covered with plastic bags, sealed with rubber bands, and arranged on the bench in a randomized complete block design with seven replications per treatment. Pots were uncovered 10 days after treatment. At 11 days after treatment, an aliquot of approximately 40 morningglory seeds was spread evenly over the soil surface of each pot and then covered with 100 cm³ of sand. Seed aliquots were prepared by delivering morningglory seeds into plastic cups using a precision instrument designed to measure gun powder. The number morningglory seedlings were counted 4, 10, 20, and 25 days after planting. The morningglory plants were cut at the soil surface 25 days after planting and the weight of the fresh foliage was recorded.

Results:

The number of morningglory plants in pots treated with 10 - 30 mg acrolein/kg soil tended to be less than the controls (Fig. 37A). Pots treated with 40 and 50 mg acrolein resulted in improved morningglory germination and survival; although, these results were not significant (Fig. 37A). Fresh weights of the foliar portion of the plants tended to be less in pots treated with 10 - 30 mg acrolein than the controls, conversely, pots treated with 40 and 50 mg acrolein tended to weigh more than the controls (Fig. 37A).

Conclusions:

The 10 - 30 mg range of acrolein decreased the number of morningglories while the 40 and 50 mg rates resulted in increased number of morningglories suggesting that acrolein can be used to control soil-borne seedling diseases.

Microbiology Experiment 4: The experiment was conducted to determine the relationship between drench-applied acrolein and soil-borne seedling diseases. This bioassay experiment used higher rates of acrolein than in Microbiology Experiment 3.

Materials and Methods:

Soil for the experiment was the same as in Microbiology Experiment 3. Acrolein was delivered in 100 ml final volume at rates: 10, 20, 30, 40, 50, 60, 80, 100, 120, 140, 160, and 200 mg/kg soil prepared from a stock 1% solution. Two water-treated controls were included. Pots were covered and arranged as described in Microbiology Experiment 3. At 13 days after treatment, pots were uncovered, approximately 40 morningglory seeds (aliquot of seeds described in Microbiology Experiment 3) were spread evenly over the soil surface of each pot, and the seeds were covered by 100 cm³ of sand. The number of morningglory plants was counted 6, 12, 19, and 27 days after planting. The morningglory plants were removed from the pots 27 days after planting, the weights of fresh shoots and roots were recorded, and ratings for root condition (rating scale 1 - 5; 1= best, 5= worst) and root disease index (rating scale 0 - 5; 0= no disease, 5= maximum disease) were determined. Pots were planted with five cotton seeds per pot one day after the morningglory plants were taken out (28 days after treatment). Cotton was allowed to grow for 20 days at which point they were removed from the soil and data

were taken on: the number of plants per pot, height of shoots (centimeters), weight of fresh shoots (grams), weight of fresh roots (grams), root condition ratings were made (rating scale 1 - 5; 1= best, 5= worst), and root disease index ratings were made (rating scale 0 - 5; 0= no disease, 5= maximum disease).

Results:

Pots treated with 20, 40, 50, and 60 mg acrolein had more morningglory seeds germinating and surviving per pot than the controls; all other treatments were similar to the controls in the first three morningglory counts (Fig. A). By the fourth count, there were no significant differences among treatments but the 20, 40, 50, and 60 mg acrolein-treated pots still tended to have more plants per pot than the controls (Fig. A). Foliar weight was improved by the 20, 40, 50, and 60 mg rates of acrolein (Fig. A). Only pots treated with 50 mg acrolein had heavier morningglory roots than roots from the controls (Fig. B). Morningglory plants removed from pots treated with 40 - 60 and 120 - 200 mg acrolein had improved root condition ratings, and pots treated with 30, 40, 60, and 100 - 200 mg acrolein resulted in improved root disease index ratings (Fig. B). There were no differences in the number of cotton plants, shoot heights, or shoot weights (Fig. C). Also, there were no differences in fresh root weights for any treatment; however, pots treated with ≥ 80 mg acrolein had better root condition and those with ≥ 60 mg acrolein had better root disease index ratings than the controls (Fig. D).

Conclusions:

Acrolein at 30 - 50 mg/kg soil can be used to improve seedling germination and survival suggesting that the compound may be controlling soil-borne seedling diseases.

Microbiology Experiment 5: A third bioassay experiment was conducted to confirm results from Microbiology Experiments 3 and 4.

Materials and Methods:

The experiment was setup and treated exactly as described in Microbiology Experiment 4. Pots were uncovered 13 days after treatment when weeds were counted, and *Trichoderma* spp. on the soil surface was rated (rating scale 0 - 5; 0= no *Trichoderma* spp., 5= maximum *Trichoderma* spp.). Soil was then removed from the first four replications of pots treated with 20, 40, 60, 100, 140, 160, and 200 mg acrolein as well as from those treated with water only. The soil was placed in a plastic bag, shaken to homogenize, and 50 cm³ was taken for fungal plating; the remaining soil was placed back into the pot. Plating methods and results are presented in Microbiology Experiment 6. Morningglory seeds were planted as for Microbiology Experiment 3. The number of morningglory plants was determined 3, 10, 15, and 23 days after planting. Morningglory plants were removed 23 days after planting, the weights of fresh shoots and roots were recorded, and root condition ratings (rating scale 1 - 5; 1= best, 5= worst) and root disease indices (rating scale 0 - 5; 0= no disease, 5= maximum disease) were recorded. Pots were planted with cotton (five seeds per pot) the same day the morningglories were removed. Cotton was allowed to grow for 20 days at which point the plants were removed from the soil and data were collected on: the number of plants per pot, height of shoots, weights of fresh shoots and roots, and root condition ratings (rating scale 1 - 5; 1= best, 5= worst), and root disease indices were determined (rating scale 0 - 5; 0= no disease, 5= maximum disease).

Results:

Some large crabgrass, pigweed, morningglory, and yellow nutsedge plants were noted in pots treated with ≤ 60 mg acrolein (Fig. 39A). These numbers were significantly diminished in pots treated with 50 mg acrolein and those treated with 60 mg acrolein had populations statistically similar to pots with none (Fig. 39A). *Trichoderma* spp. were noted on the soil surface of some of the pots treated with ≥ 80 mg acrolein; all pots treated with ≥ 100 mg acrolein had higher *Trichoderma* spp. ratings than all other treatments (Fig. 39B). In the first morningglory count, only pots treated with 140 mg acrolein had improved morningglory germination and survival (Fig. 39C). By the second and third morningglory counts, pots treated with 100, 140, 160, and 200 mg acrolein had more morningglories than the controls (Fig. 39C). In the last morningglory count, pots treated with ≥ 140 mg acrolein had the most morningglory plants per pot (Fig. 39C). Pots treated with 30, 50, 80, and 120 mg acrolein had improved root condition ratings (Fig. 39D). The fresh weights of the morningglory roots from pots treated with ≥ 80 mg acrolein were heavier than the roots from the control pots (Fig. 39D). Morningglories from pots treated with 140 and 160 mg acrolein were taller than the controls, and those from pots treated with 10 mg acrolein were shorter than the controls (Fig. 39E). Cotton root weights were improved in soils treated with 120, 160, and 200 mg acrolein (Fig. 39F). Root condition was improved for cotton plants grown in all acrolein-treated pots except those treated with 10 and 40 mg acrolein (Fig. 39F). Disease index ratings were better for cotton in acrolein-treated soils except those treated with 40 mg (Fig. 39F). There were no differences in the number of cotton plants, the weight of fresh shoots, or the height of shoots for any treatment (Fig. 39G).

Conclusions:

Increasing rates of acrolein resulted in: better weed control, increased *Trichoderma* spp. incidence, improved morningglory germination and survival, heavier morningglory and cotton roots, and improved appearance of morningglory and cotton roots.

Microbiology Experiment 6: A microbial plating experiment was conducted to determine the effects of drench-applied acrolein on the soil microflora using media specific for fungi, bacteria, and actinomycetes.

Materials and Methods:

The experiment was conducted using the 50 cm³ soil samples that were collected from Microbiology Experiment 5 and Nematode Experiment 7 (preparation, experimental design, and treatment methods described in those sections). Soil samples from Microbiology 5 were collected from pots treated with 20, 40, 60, 100, 140, 160, and 200 mg acrolein as well as from the water-treated controls. For microbial plating, 10 gram sub-samples were taken from the homogenous 50 cm³ samples. The 10 gram sub-samples were each added to 250 ml Erlenmeyer flasks, each containing 98 ml of sterile, and blended with a magna-stirrer. While vortexing, a second dilution was made by removing five ml from each suspension and delivering them into 500 ml Erlenmeyer flasks with each with 340 ml sterile water in each (Johnson and Curl, 1972). Also while the initial dilution was still vortexing, individual drops were removed one at a time and delivered into Petri plates and aluminum weighing dishes using a small, sterile Pasteur pipette. Only one drop of the suspensions was delivered to each of five Petri plates per

trial replication. Ohio agar (Johnson and Curl, 1972) was poured over the suspension drops in the plates until approximately 75% of the plate was covered with agar and the plates were gently swirled by hand to evenly distribute the soil suspension in the agar medium. The aluminum weighing dishes received 20 drops of the suspension, each drop taken from the suspension one at a time. The aluminum weighing dishes were numbered, dried for 24 hours in an oven set at 100°C, and the dry weights were recorded prior to receiving the soil suspension drops. After receiving 20 drops of the initial soil suspension, the aluminum weighing dishes were again placed in an oven set at 100°C for 24 hours to dry. Dry weights of the dishes with dried soil were recorded and the weight of soil per drop was calculated for determining the number of microorganisms per gram of soil. From the second dilutions (in the 500 ml Erlenmeyer flasks), one drop was delivered to each of 10 Petri plates, five of which received Thornton's standardized agar and the other five received Benedict agar (Johnson and Curl, 1972). The plates were swirled to distribute the soil suspension throughout the agar media. Media, procedures, and methods are detailed in the appendix.

The antibiotics used in the Benedict's agar were old and resulted in an inadequate medium for actinomycetes plating. A similar procedure for plating actinomycetes was repeated using soil collected from Nematode Experiment 7. The only changes in the actinomycetes plating procedure was the soil dilution for this test was prepared by taking a two gram sub-sample from the 50 cubic centimeter soil sample and suspending it in 98 ml sterile water.

All plates were stacked, placed into plastic sleeves, and incubated at room temperature in a closed cabinet. Fungal and bacterial colonies were counted three and 8

days after plating and actinomycetes colonies were counted at 4 and 9 days. Few differences were noted between first and second plate counts therefore only the final count shall be presented.

Results:

The lowest rate of acrolein stimulated bacterial populations while rates ≥ 140 mg acrolein/kg soil had fewer bacteria per gram of soil than the controls (Fig. 40A). A trend of stimulation at lower rates followed by a reduction in bacterial populations as rates of acrolein were increased was apparent (Fig. 40B). In contrast, while only the highest rate of acrolein had statistically greater actinomycetes populations, a clear trend of increase in populations as rates of acrolein were increased was observed (Fig. 40C). A similar trend was also observed with number of fungi increasing as the rates of acrolein increased (Fig. 40D). Pots treated with 100 and 140 mg acrolein had more fungal colonies than those treated with lower rates of acrolein as well as the controls (Fig. 40E). Pots treated with 160 and 200 mg acrolein had greater fungal counts than all other treatments (Fig. 40E). Furthermore, while the number of fungal colonies increased, diversity decreased to primarily one fungal genus: *Trichoderma*. The *Trichoderma* data from the soil plating followed a similar trend to results taken on visual rating of *Trichoderma* presence on the surface of the soil following acrolein treatments (see Microbiology Experiment 5, Fig. 39B). As the rate of acrolein applied to the soil increased, morningglory germination and survival increased (Microbiology Experiment 5, Fig. 39C).

Conclusions:

As rates of acrolein are increased, the number of bacteria per gram of soil decreased while the number of actinomycetes and fungi increased. Acrolein treatments stimulate *Trichoderma* spp.

Microbiology Experiment 7: The experiment was designed to determine the effect of drench-applied acrolein on soil enzymatic activities.

Materials and Methods:

The experiment was setup using Tallassee field soil mixed 50:50 (v/v) with sand in 1 kg amounts in 10 cm diameter PVC pots on a greenhouse bench. Acrolein treatments were drench-applied in a final volume of 100 ml per pot at rates of 10, 20, 30, 40, 50, 60, 80, 100, 120, 140, 160, and 200 mg/kg soil prepared from a stock 1% acrolein solution. Controls were treated with 100 ml water only. Pots were covered with HDPE plastic bags immediately following treatment, were sealed with rubber bands, and were arranged on the greenhouse bench in a randomized complete block design with seven replications per treatment. Pots were uncovered 10 days after treatment, soil samples were taken, and five 'Marketmore 76' cucumber (*Cucumis sativus* 'Marketmore 76') seeds were planted per pot. Cucumbers were allowed to grow for 52 days at which time the experiment was taken down and final soil samples were taken. Pre-plant and final soil samples (50 cm³) were spread out on plastic weighing-dishes for air-drying immediately after collection and air-dried at room temperature (20 - 22°C) for 3 - 5 days. The dried samples were sieved through a 1mm mesh screen, placed in sealable plastic bags, and stored at <0°C. Enzymatic activities analyzed were: catalase, α -galactosidase,

β -galactosidase, α -glucosidase, β -glucosidase, chitinase, sulfatase, phosphatase, and urease using methods published by Rodríguez-Kábana and Truelove (1970), Rodríguez-Kábana and Truelove (1982), Rodríguez-Kábana *et al* (1983), Alef and Nannipieri (1995), and Quiroga-Madrigal (2000). Details of the procedures used are included in the appendix of this dissertation.

Results:

Catalase

Catalase activity increased in response to 10 mg treatments of acrolein and decreased with the 50 and 60 mg treatments of acrolein (Fig. 41A). In the final sample, after 52 days with cucumber plants, soil from pots treated with acrolein evidenced increased catalase (Fig. 41A).

α -galactosidase

Soil samples collected prior to planting evidenced decreasing α -galactosidase activity as the rates of acrolein increased (Fig. 41B). All rates > 20 mg/kg soil had significantly lower activity than the controls. Soils treated with acrolein at rates 140, 160, and 200 mg/kg soil had lower activity than all other treatments ≤ 100 mg (Fig. 41B). All acrolein treated-soil had higher activity in the final soil sample than in the pre-plant sample; activity in the controls did not increase by the final sample. Activity of α -galactosidase in the final soil sample was greater in pots treated with 10 and 20 mg acrolein/kg soil than the controls (Fig. 41B).

β -galactosidase

In the initial soil sample, pots treated with ≥ 40 mg acrolein had reduced β -galactosidase activity evidencing a trend of decreasing activity as acrolein rates were

increased (Fig. 41C). β -galactosidase activity increased for each treatment from the first soil sample to the final sample (Fig. 41C). Acrolein at rates of 50 and ≥ 80 mg reduced β -galactosidase activity in the final soil sample (Fig. 41C). Again, a trend of decreasing activity with increasing rates of acrolein was apparent in the final soil sample (Fig. 41C).

α -glucosidase

In the initial soil sample, all pots treated with 60 - 100 mg acrolein had lower α -glucosidase activity than the controls (Fig. 41D). All pots treated with 120 - 160 mg acrolein had lower activity than those treated with lower rates, and pots treated with 200 mg acrolein had lower α -glucosidase activity than all other treatments (Fig. 41D). α -glucosidase activity of all acrolein treatments ≤ 140 mg was lower in the second soil sample than the initial sampling (Fig. 41D). In the final soil sample, α -glucosidase activity was lower in all acrolein treatments than the controls (Fig. 41D). Acrolein treatments ≥ 100 mg acrolein resulted in lower activity than all other treatments and acrolein at rates 160 and 200 mg acrolein had the lowest activity (Fig. 41D).

β -glucosidase

Acrolein at 10 mg/kg soil stimulated β -glucosidase activity in the pre-planting soil sample (Fig. 41E). All other acrolein treatments had lower activity than the controls and all soils treated with ≥ 60 mg acrolein had lower β -glucosidase activity than all other treatments (Fig. 41E). β -glucosidase activity in the final soil samples was higher than in pre-planting samples (Fig. 41E). In the final soil sample, pots treated with ≥ 100 mg acrolein had lower β -glucosidase activity than the controls and those treated with 140, 160, and 200 mg had the lowest activity (Fig. 41E).

Chitobiase

All pots treated with ≥ 20 mg acrolein had lower chitobiase activity than the controls in the initial soil sample (Fig. 41F). Activity from all treatments was higher in the final soil sample than in the initial sample (Fig. 41F). In the final sample, all pots treated with ≥ 20 mg acrolein had reduced chitobiase activity in a pattern typical of a logistic dose response (Fig. 41G).

Phosphatase

All pots treated with ≥ 30 mg acrolein evidenced reduced phosphatase activity (Fig. 41H). Phosphatase activity increased in most treatments by the final soil sample. In the final sample, phosphatase activity was higher in the controls than in all other treatments (Fig. 41H). Phosphatase activity in pots treated with 10 mg acrolein was higher than the activity in all other pots treated with higher rates of acrolein (Fig. 41H). The trend for phosphatase activity in the final sample decreased with increasing doses following a logistic model (Fig. 41I).

Sulfatase

In the initial soil sample, only pots treated with 200 mg acrolein had lower sulfatase activity (Fig. 41J). At the final sample, only the controls and pots treated with 200 mg acrolein had higher sulfatase activity than at the first soil sample; all other acrolein treated-pots had lower activity in the final soil sample (Fig. 41J). In the final soil sample, all pots treated with acrolein except the 10 mg rate evidenced reduced sulfatase activity (Fig. 41J). There was a trend of lower activity with increasing rates of acrolein in the final soil sample (Fig. 41J).

Urease

There were no significant differences in urease activity in the pre-planting soil sample (Fig. 41K). In the final sample, all pots treated with acrolein except the 20 and 30 mg rates had significantly lower urease activity as compared to the controls (Fig. 41K).

Conclusions:

In general, as rates of acrolein were increased, most of the enzymatic activities decreased. This is indicative of biocidal activity. With the exception of sulfatase, enzymatic in the final soil samples was lower than activity in the controls; activities decreased as rates of acrolein increased.

Microbiology Experiment 8: An experiment was conducted to measure the effects of drench-applied acrolein on soil pH and electrical conductivity (EC).

Materials and Methods:

The soil used for pH and (EC) determination was from Microbiology Experiment 7. Experiment, preparation, design, and soil sampling are detailed in Microbiology Experiment 7. For soil pH determination, five grams of the air-dried soil sample was weighed into 30 ml plastic cups, 10 ml of demineralized water was added, and the cups were shaken on a mechanical shaker for 10 minutes. The soil slurry was stirred again with a plastic coffee stirrer just before measuring pH potentiometrically with a Fisher Scientific, Accumet Research, AR10 ph meter (Klute, 1986). The supernatant from the cups was then poured into a 10 ml polypropylene centrifuge tube and centrifuged at $\frac{3}{4}$ speed for 20 - 25 minutes (until clear). Soil electrical conductivity

(EC) was then determined with a Industrial Instruments Inc. Model RC 16B2 conductivity bridge (Klute, 1986).

Results:

All soils treated with 40 - 60 and 140 - 200 mg acrolein had lower pH than those of the controls in the pre-planting soil sample (Fig. 42A). All treatments ≥ 20 mg acrolein, except the 140 mg treatment, resulted in soils with lower pH than the controls in the final soil sample (Fig. 42A). In the pre-plant soil sample, pots treated with 20, 50, 160, and 200 mg acrolein/kg soil had higher soil electrical conductivity than the controls (Fig. 42B). Electrical conductivity was lower in the final soil samples from pots treated with 10, 50, 80, 120, 140, and 160 mg acrolein than the controls while all other acrolein treatments were similar to the controls (Fig. 42B).

Conclusions:

Acrolein has some effect on soil pH; however, it has less of an effect on pH than growing cucumbers in the pots for 52 days. Acrolein tended to increase soil EC as rates were increased; after 52 days of cucumber plants the soil EC was lowered.

D. DISCUSSION

Results from dipping caladium bulbs show that acrolein may be useful for disinfesting plant materials prior to planting. Disinfesting plant material and bulbs prior to planting is an effective means of disease prevention in several agricultural industries including ornamentals, potato, and banana production. Low concentrations of acrolein for dipping times of less than 2 minutes were the most effective at improving plants.

This use of acrolein is not practical for methyl bromide as it has little solubility in water and would most likely dissipate too quickly.

Bioassay experiments suggest that acrolein is effective at controlling soil-borne seedling diseases. Rates of acrolein ≤ 30 mg acrolein were not able to control seedling diseases; however, ≥ 40 mg acrolein increased the number of test plants that germinated and survived. These higher rates also resulted in taller, heavier, and healthier plants than the controls. Increased incidence of *Trichoderma* spp. on the surface of the soil was observed with effective rates of acrolein. Drench applications of acrolein result in soils that are less conducive for soil-borne seedling diseases. This is very significant for agricultural use because seedling diseases can be extremely problematic in many agronomic and horticultural crops. Since no phytotoxicity was observed, and plant growth parameters measured were all improved with these rates, acrolein may be useful for disinfecting agricultural soils prior to planting.

Results from soil enzymatic activity determinations showed that as rates of acrolein are increased, enzymatic activities of the soil generally decrease. This is common to many broad-spectrum biocides that are used to sterilize soil. When microbial plating was conducted with acrolein-treated soils, it was apparent that acrolein was not acting as a biocide, rather it was acting as a selective pesticide reducing some groups of organisms while stimulating others. Bacteria of the soil were decreased as acrolein rates were increased, conversely, actinomycetes populations increased as the rates of acrolein treatments increased. Large populations of actinomycetes are often associated with a microflora of a "healthy soil" (van Bruggen and Semenov, 2000; Hobbs, 2007). Additionally, as the rate of acrolein treatments were increased, the number of fungal

colonies in the soil increased. Although the number of fungal colonies increased, the diversity of the fungal communities decreased to nearly one fungal genus, *Trichoderma*. This a very positive result of acrolein treatments as *Trichoderma* spp. are known to compete with soil-borne plant pathogenic microorganisms, reduce the incidence of disease, and improve plant growth (Papavizas, 1985; Windham and Elad, 1986). These results suggest that acrolein can be used to disinfest agricultural soils prior to planting while potentially improving the soil microflora so that it is not conducive for plant diseases.

Acrolein applications in the range of 40 - 200 mg/kg soil, were shown to reduce seedling diseases while improving plant growth suggesting that the chemical may be useful as an agricultural pesticide. Acrolein did not create problematic microbiological voids in the soil at these rates which is very important for effective pest control and sustainable agricultural systems. The stimulations of actinomycetes and *Trichoderma* spp. suggest that acrolein treatment may actually improve the soil microflora while controlling plant deleterious organisms.

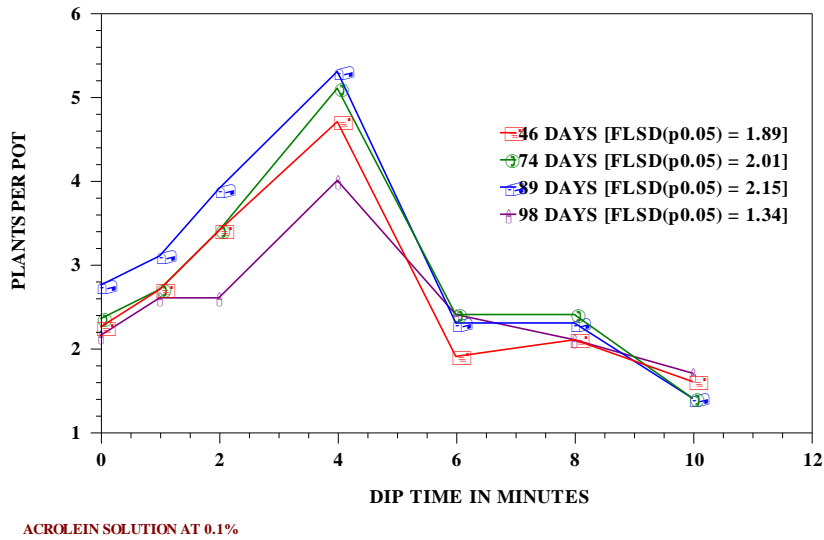


Figure 35A: Number of caladium plants per pot at 46, 74, 89, and 98 days after planting (Microbiology Experiment 1).

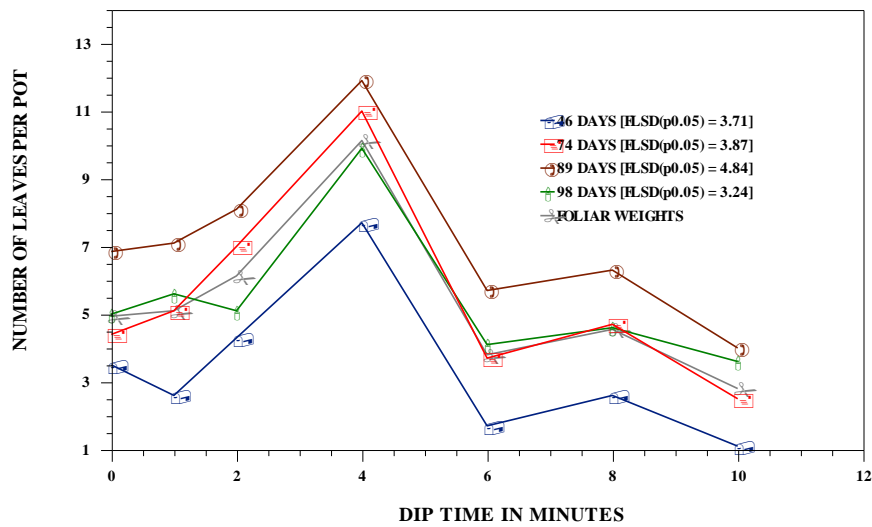


Figure 35B: Total number of leaves per pot and fresh foliar weights at 46, 74, 89, and 98 days after planting (Microbiology Experiment 1).

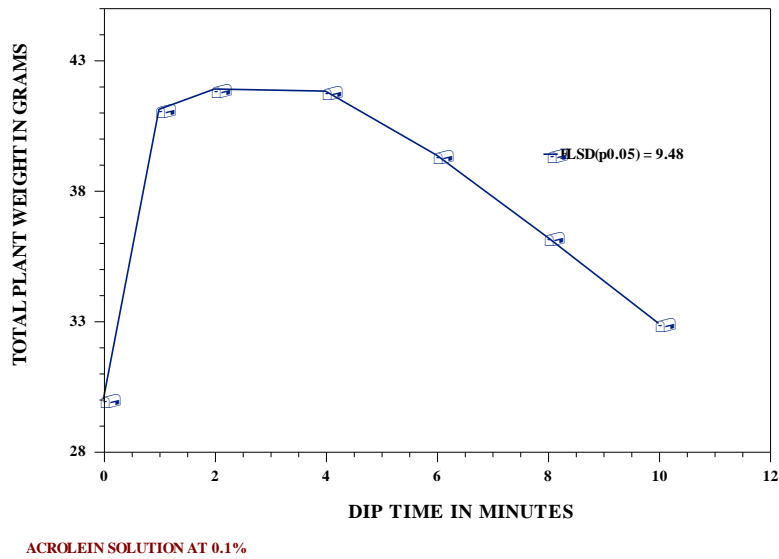


Figure 35C: Total plant weight per pot in grams (Microbiology Experiment 1).

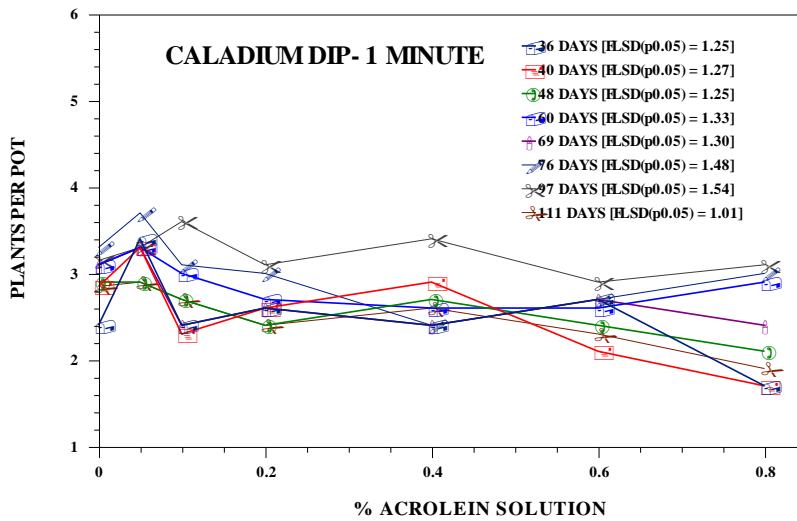


Figure 36A: Number of plants per pot from bulbs dipped for one minute (Microbiology Experiment 2).

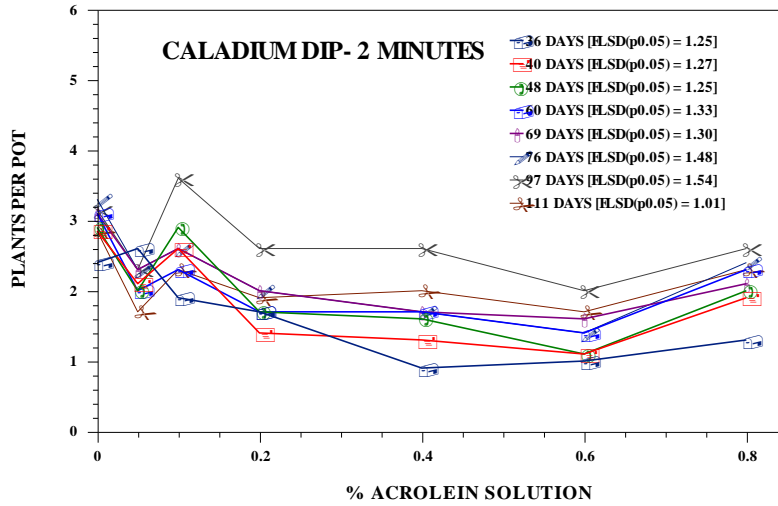


Figure 36B: Number of plants per pot from bulbs dipped for two minutes (Microbiology Experiment 2).

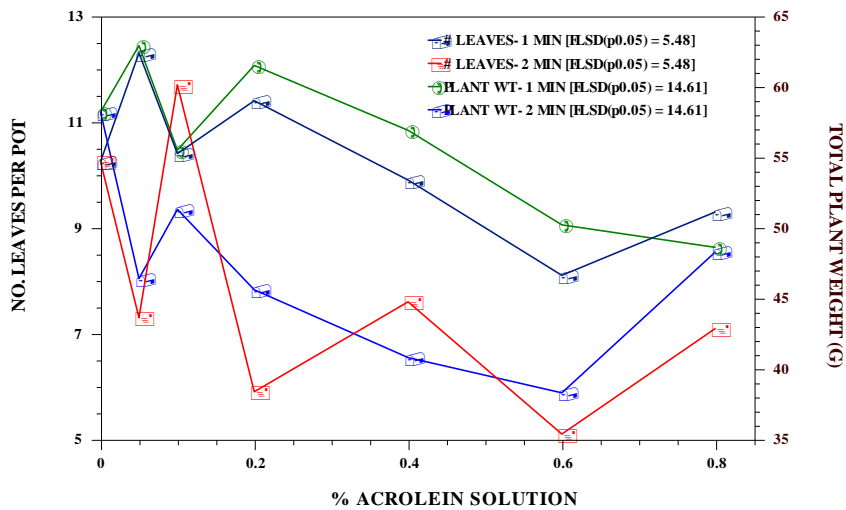
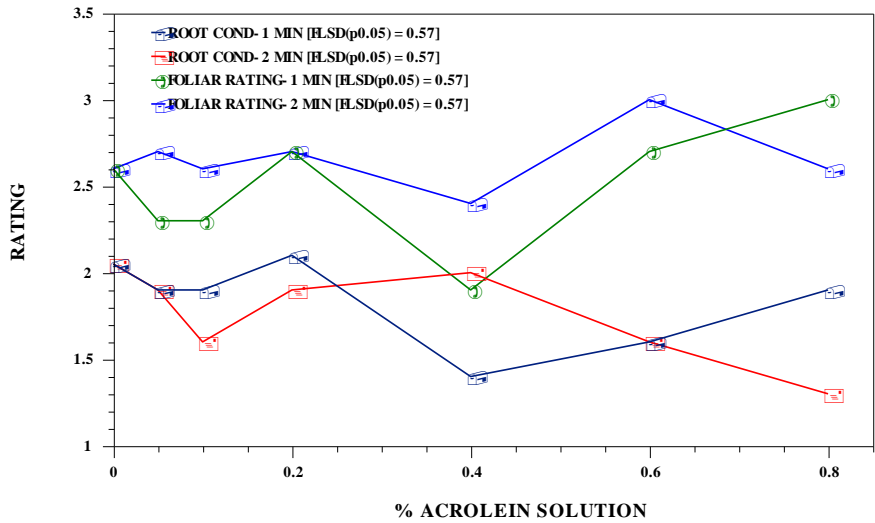


Figure 36C: Number of plants per pot and total plant weights in grams for one and two minute dips (Microbiology Experiment 2).



ROOT CONDITION SCALE: 1= BEST, 5= WORST
 FOLIAR RATING SCALE: 1= BEST, 5= WORST

Figure 36D: Root condition and foliar ratings for one and two minute dips (Microbiology Experiment 2).

ACROLEIN
 FUNGICIDE TEST VI
 12.JANUARY.2006
 TERMINATED:15.FEBRUARY.2006

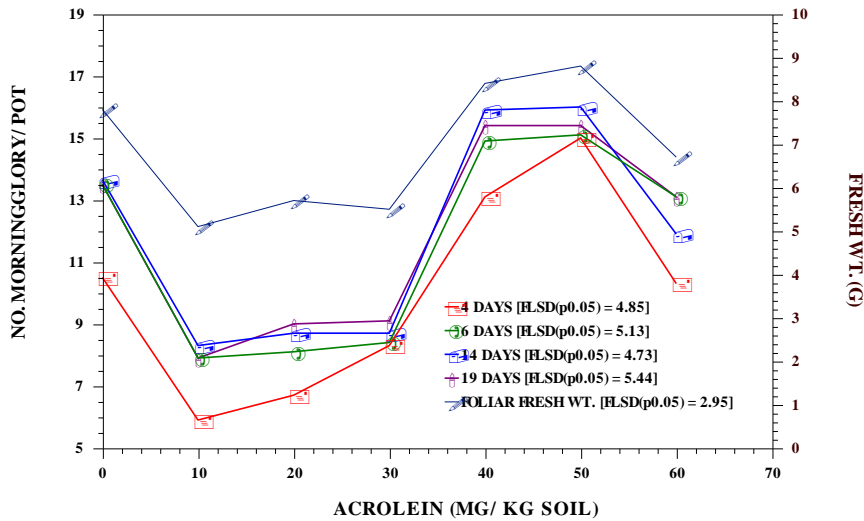


Figure 37A: Number of morningglories per pot in four weed counts and foliar fresh weights in grams (Microbiology Experiment 3).

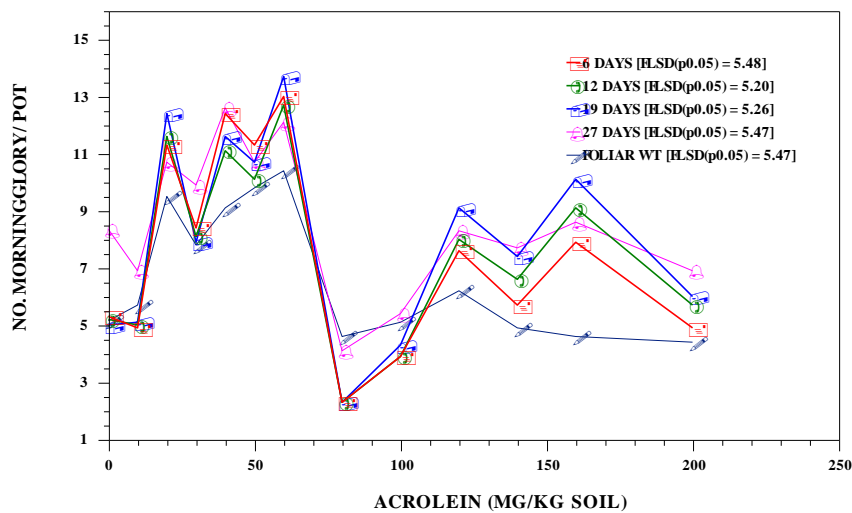
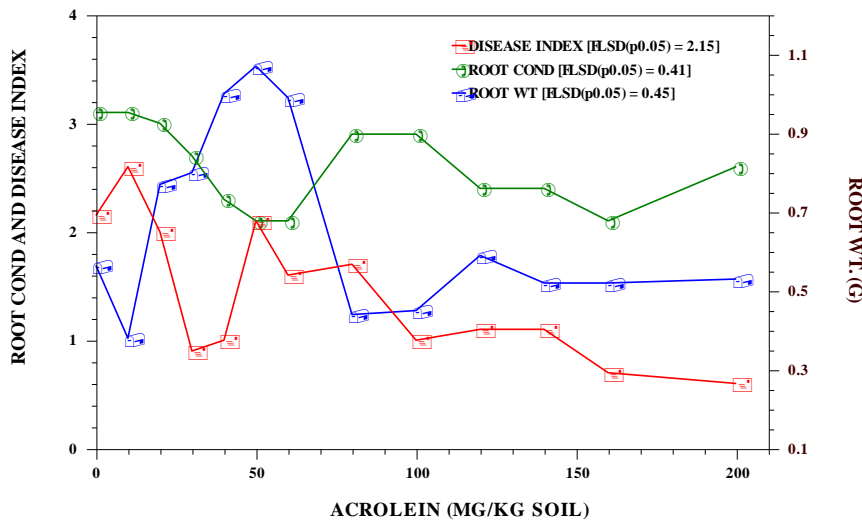


Figure 38A: Number of morningglory plants per pot in four counts and foliar fresh weight in grams (Microbiology Experiment 4).



ROOT CONDITION SCALE: 1=BEST, 5=WORST
 ROOT DISEASE INDEX SCALE: 0=NO DISEASE, 5=MAX. DISEASE

Figure 38B: Root condition and root disease index ratings and root weight in grams (Microbiology Experiment 4).

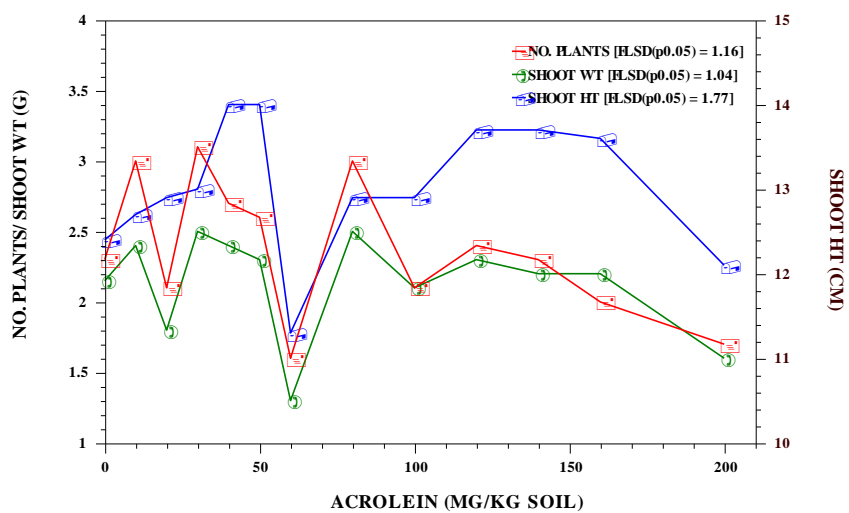
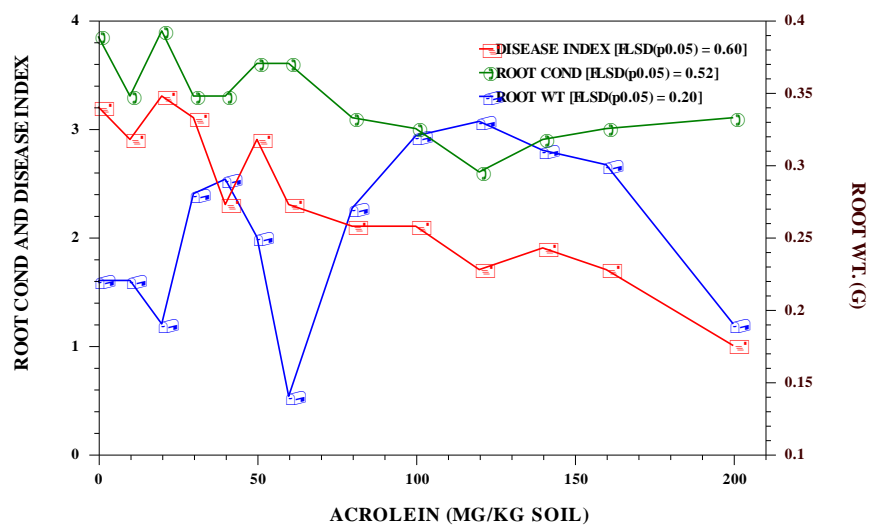


Figure 38C: Number of cotton plants, weight of shoots in grams, and heights of shoots in centimeters (Microbiology Experiment 4).



ROOT CONDITION SCALE: 1=BEST, 5=WORST
 ROOT DISEASE INDEX SCALE: 0=NO DISEASE, 5=MAX. DISEASE

Figure 38D: Cotton root condition and disease index ratings and fresh weights in grams (Microbiology Experiment 4).

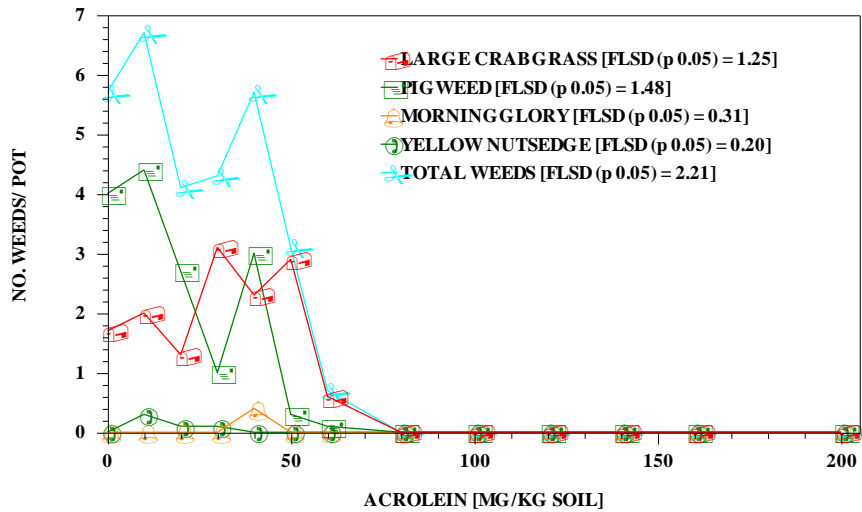
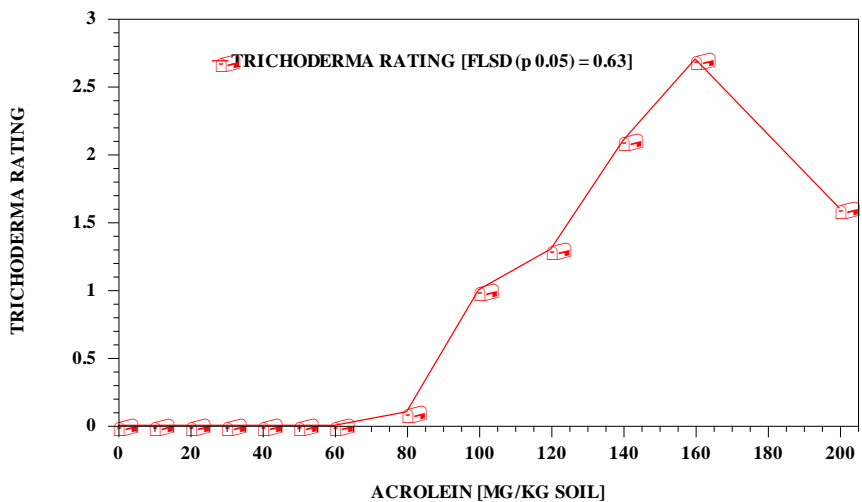


Figure 39A: Counts of the weeds which emerged after treatment (Microbiology Experiment 5).



RATING SCALE 0- 5: 0= NO TRICHODERMA, 5= MAXIMUM TRICHODERMA

Figure 39B: Ratings of *Trichoderma* spp. visible on the soil surface (Microbiology Experiment 5).

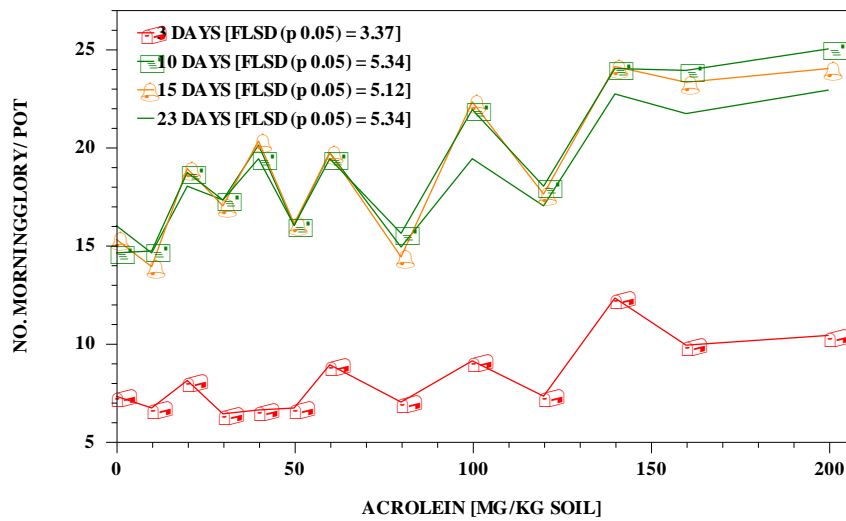
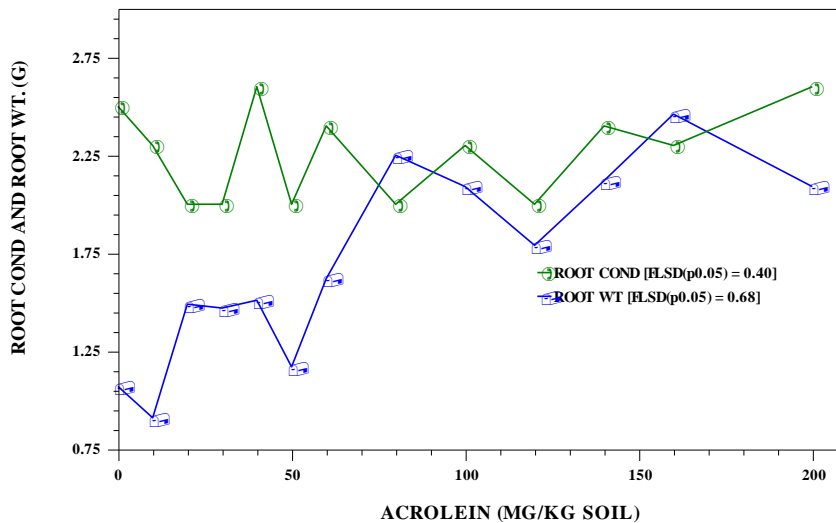


Figure 39C: Number of morningglories that germinated and survived after being planted (Microbiology Experiment 5).



ROOT CONDITION SCALE: 1=BEST, 5=WORST

Figure 39D: Morningglory root condition ratings and fresh root weights in grams (Microbiology Experiment 5).

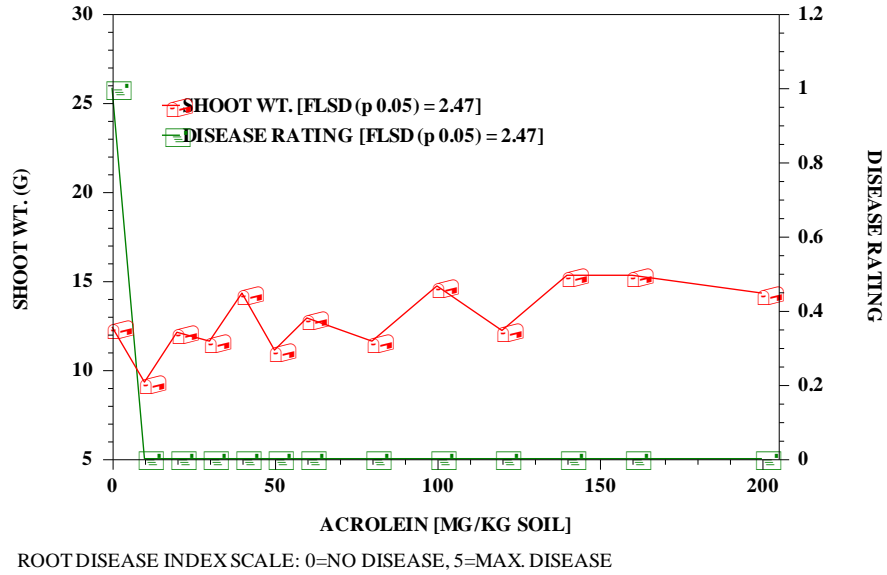


Figure 39E: Morningglory fresh foliar weights in grams and root disease ratings (Microbiology Experiment 5).

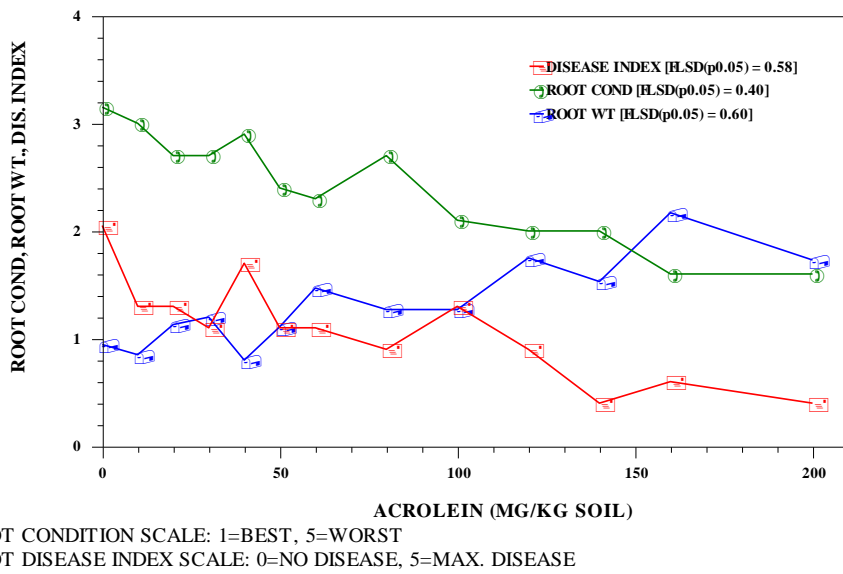


Figure 39F: Cotton root disease and condition ratings as well as fresh root weights in grams (Microbiology Experiment 5).

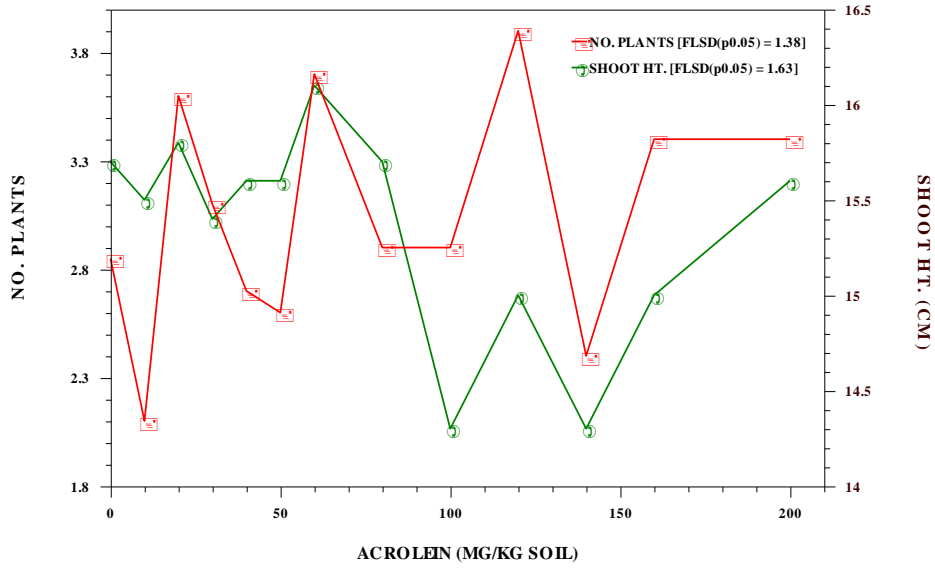


Figure 39G: Number of cotton plants per pot and their shoot heights in centimeters (Microbiology Experiment 5).

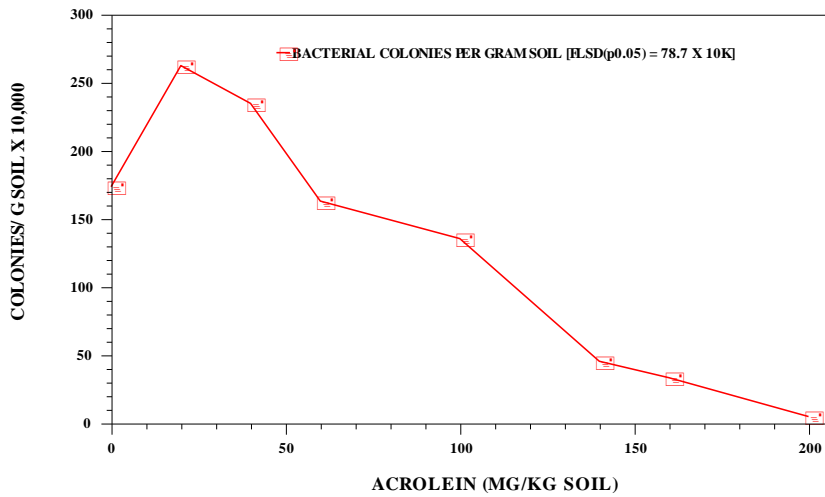


Figure 40A: Bacterial colonies per gram of soil; plated using Thornton's medium (Microbiology Experiment 6).

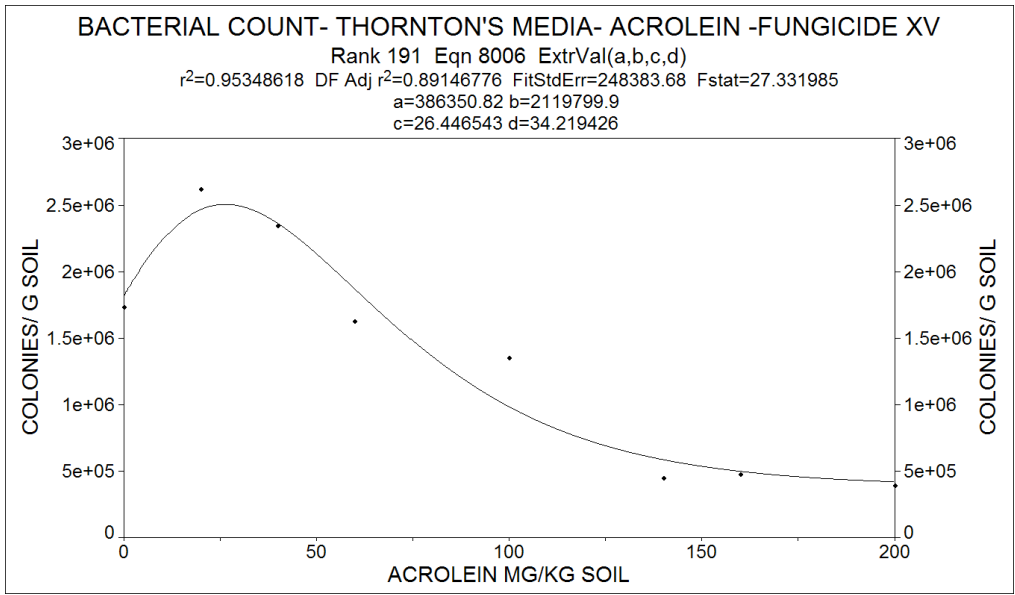


Figure 40B: Trend of the effects of acrolein on soil bacteria (Microbiology Experiment 6).

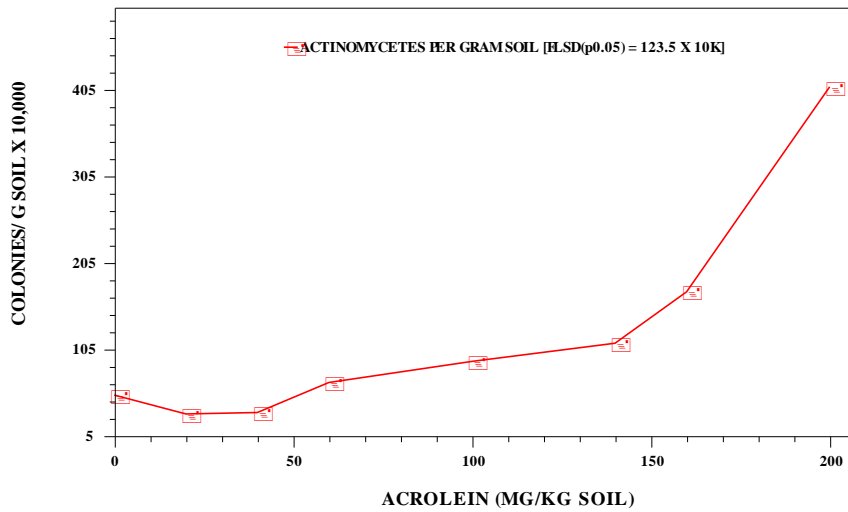


Figure 40C: Actinomycetes colonies per grams of soil; plated using Benedict's medium (Microbiology Experiment 6).

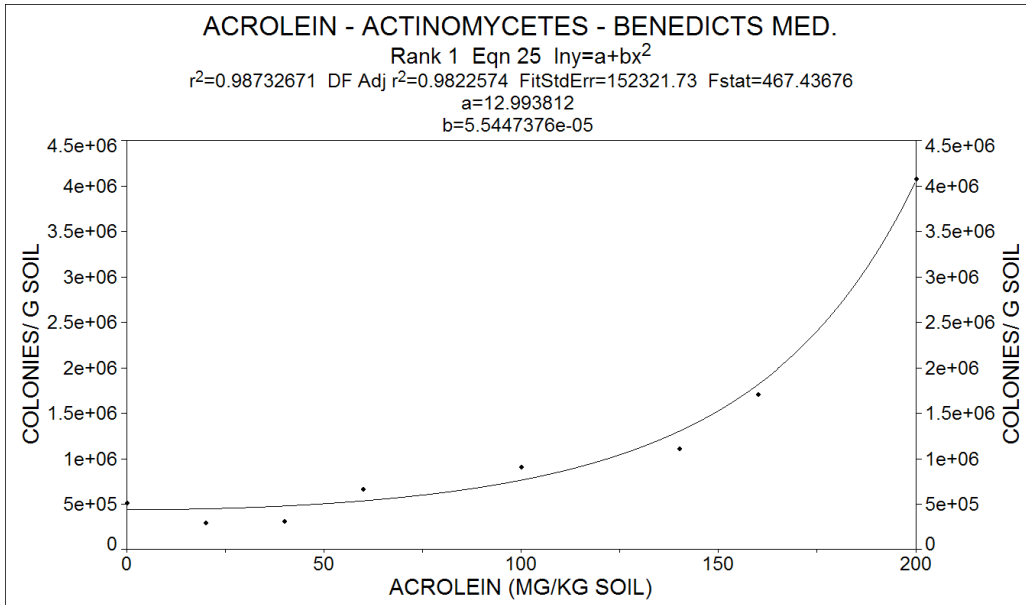


Figure 40D: Trend of the effects of acrolein on soil actinomycetes (Microbiology Experiment 6).

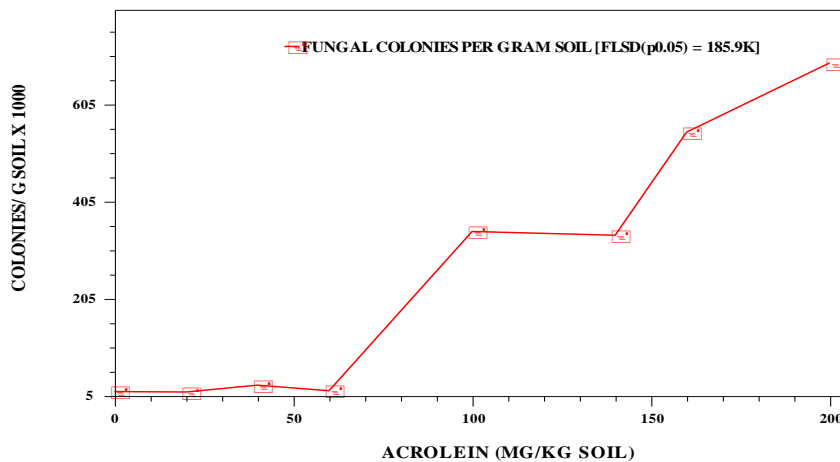


Figure 40E: Fungal colonies per gram of soil; plated using Ohio medium (Microbiology Experiment 6).

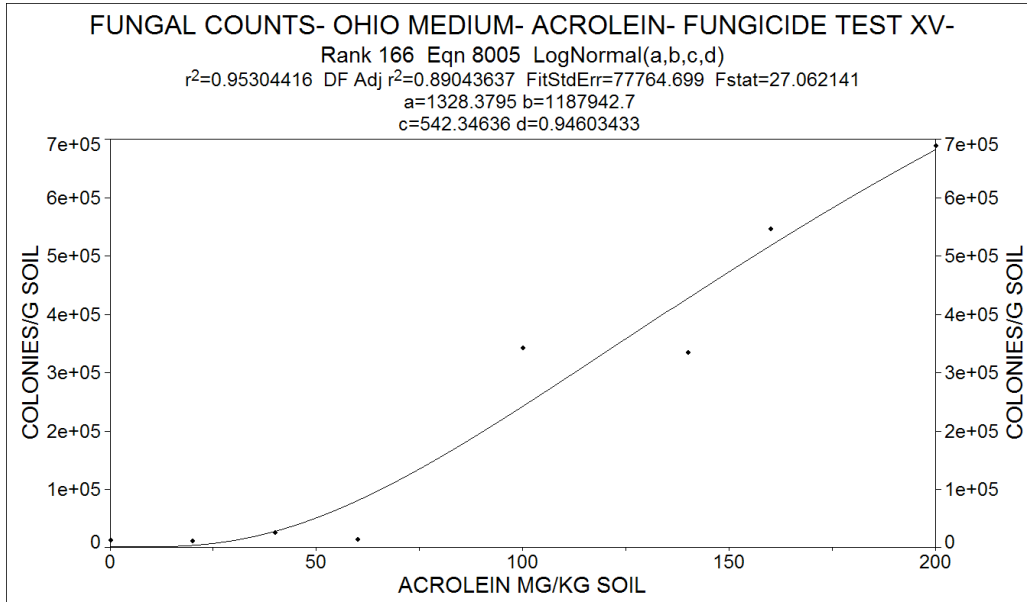


Figure 40F: Trend of the effects of acrolein on soil fungi (Microbiology Experiment 6).

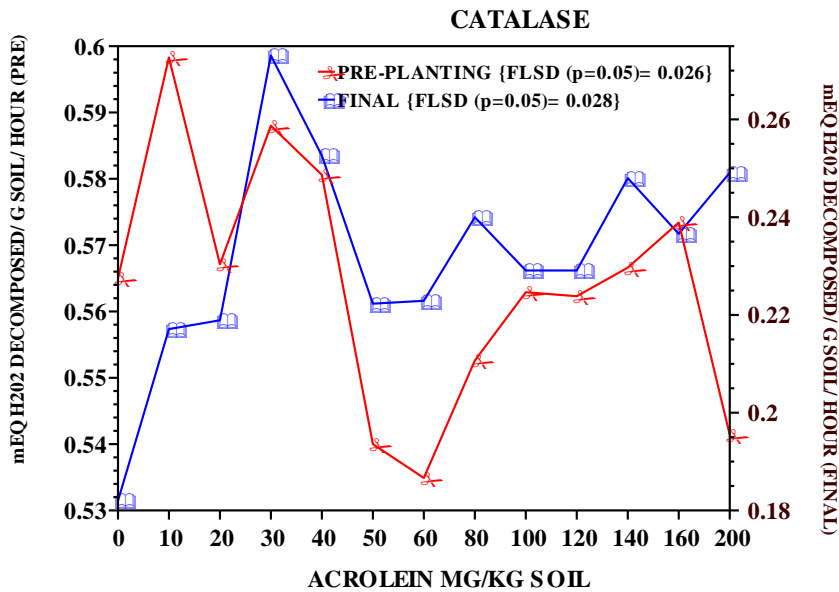


Figure 41A: Effects of drench-applied acrolein on soil catalase activity (Microbiology Experiment 7).

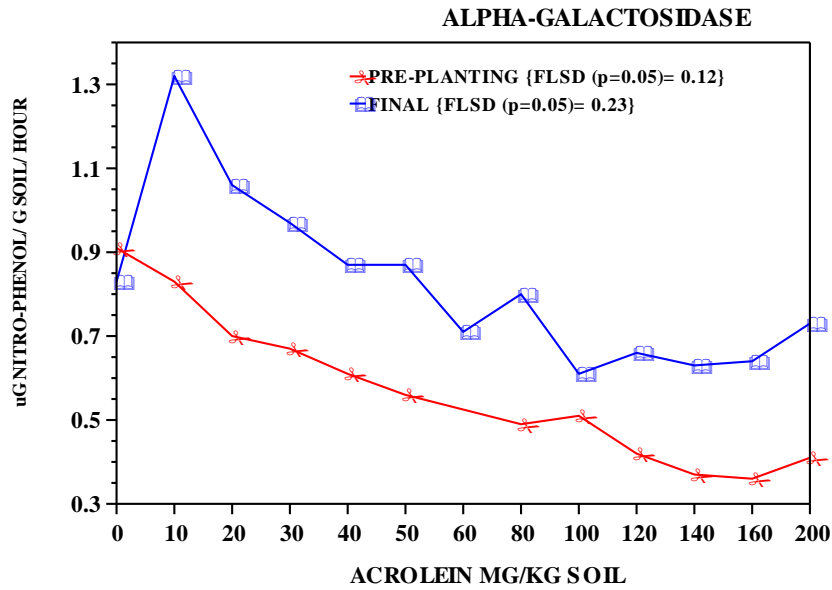


Figure 41B: Effects of drench-applied acrolein on soil α -galactosidase activity (Microbiology Experiment 7).

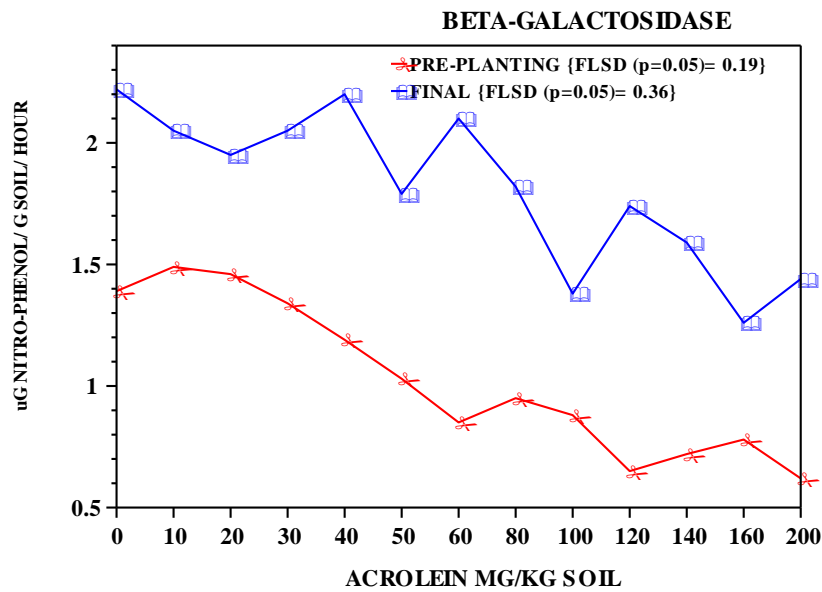


Figure 41C: Effects of drench-applied acrolein on soil β -galactosidase activity (Microbiology Experiment 7).

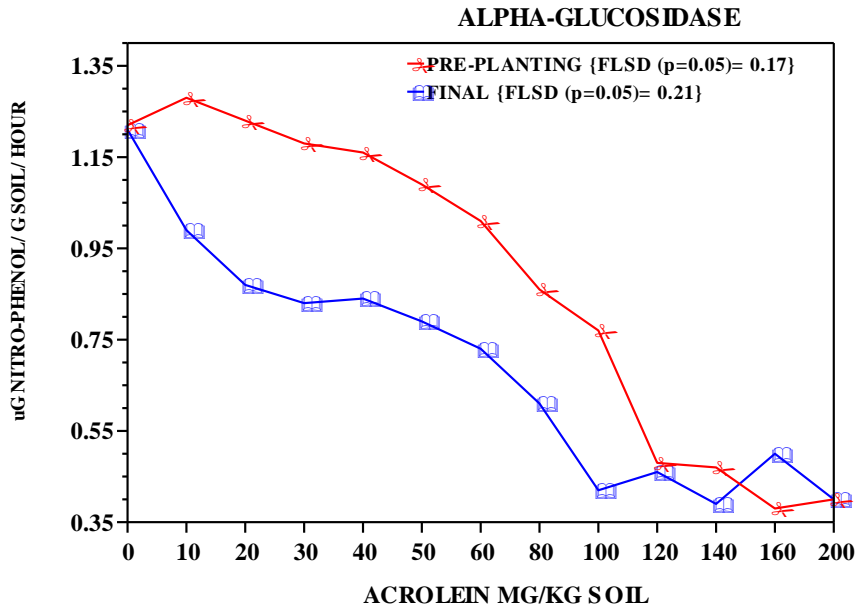


Figure 41D: Effects of drench-applied acrolein on soil α -glucosidase activity (Microbiology Experiment 7).

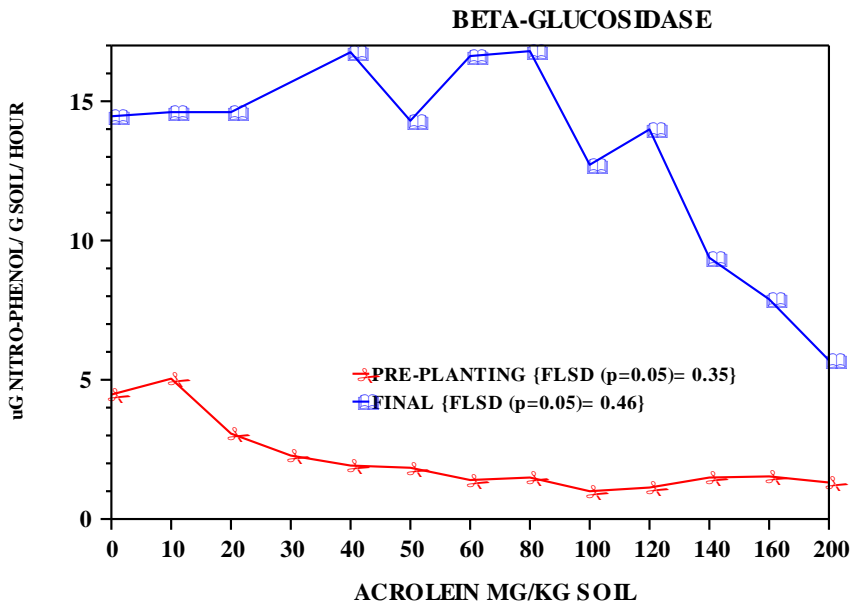


Figure 41E: Effects of drench-applied acrolein on soil β -glucosidase activity (Microbiology Experiment 7).

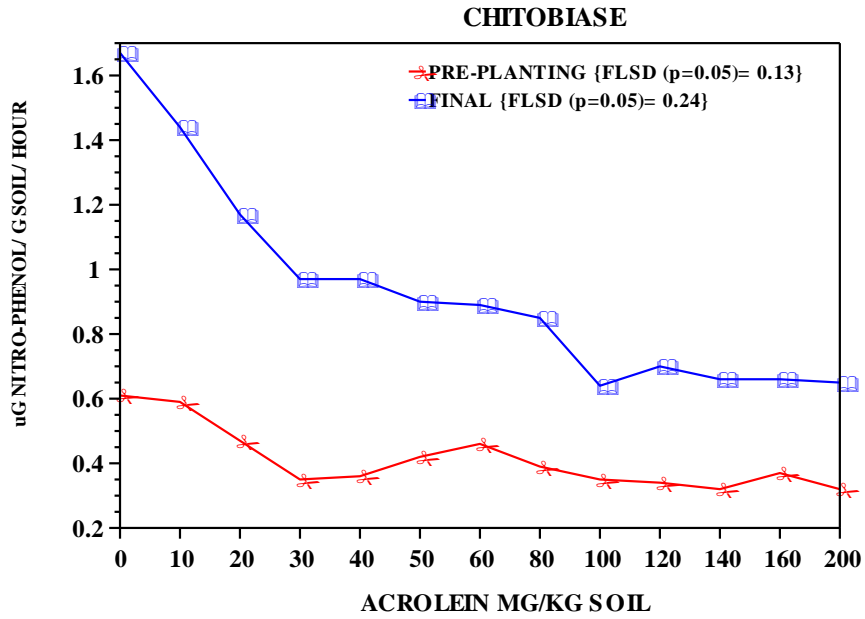


Figure 41F: Effects of drench-applied acrolein on soil chitobiase activity (Microbiology Experiment 7).

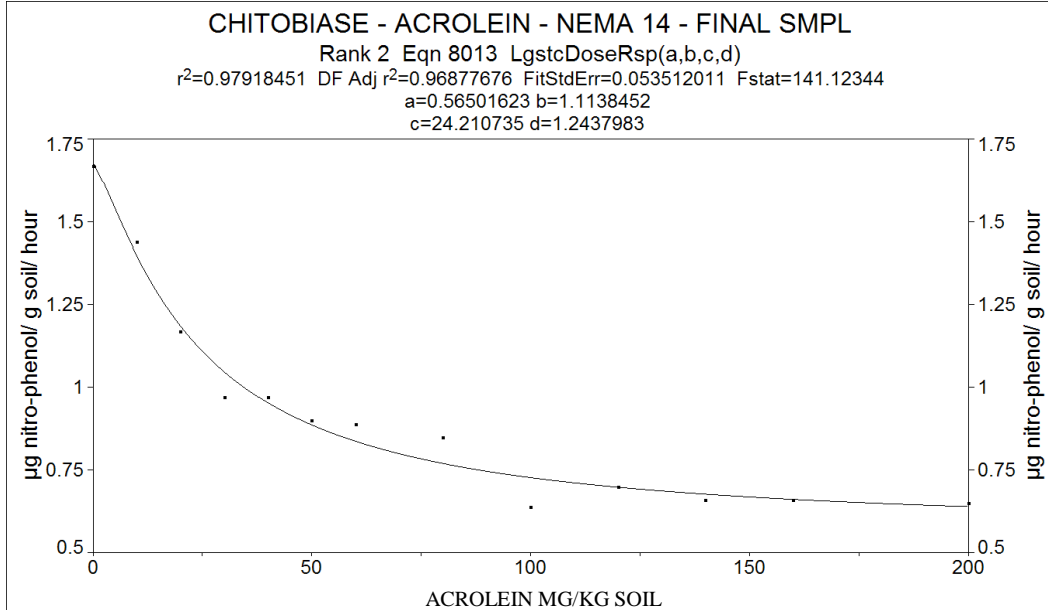


Figure 41G: Dosage response curve of soil chitobiase activity (Microbiology Experiment 7).

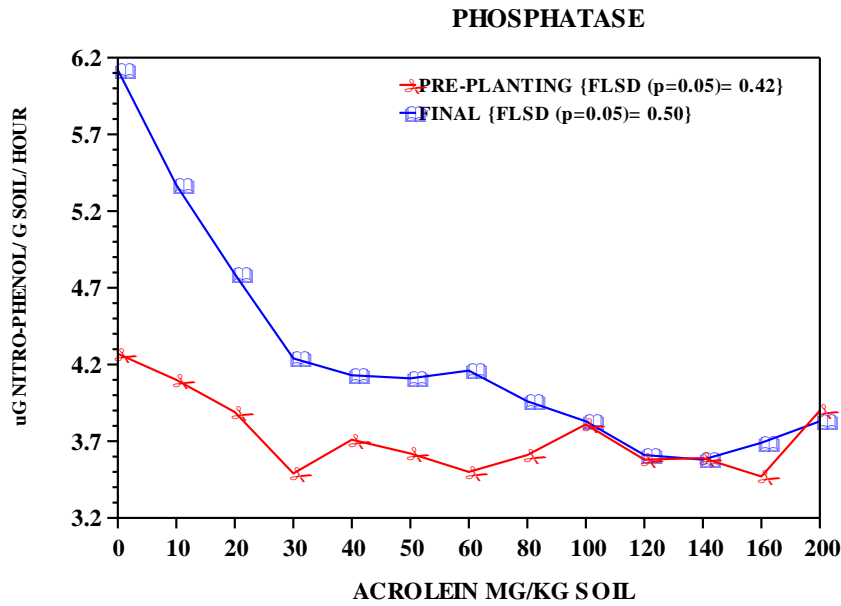


Figure 41H: Effects of drench-applied acrolein on soil phosphatase activity (Microbiology Experiment 7).

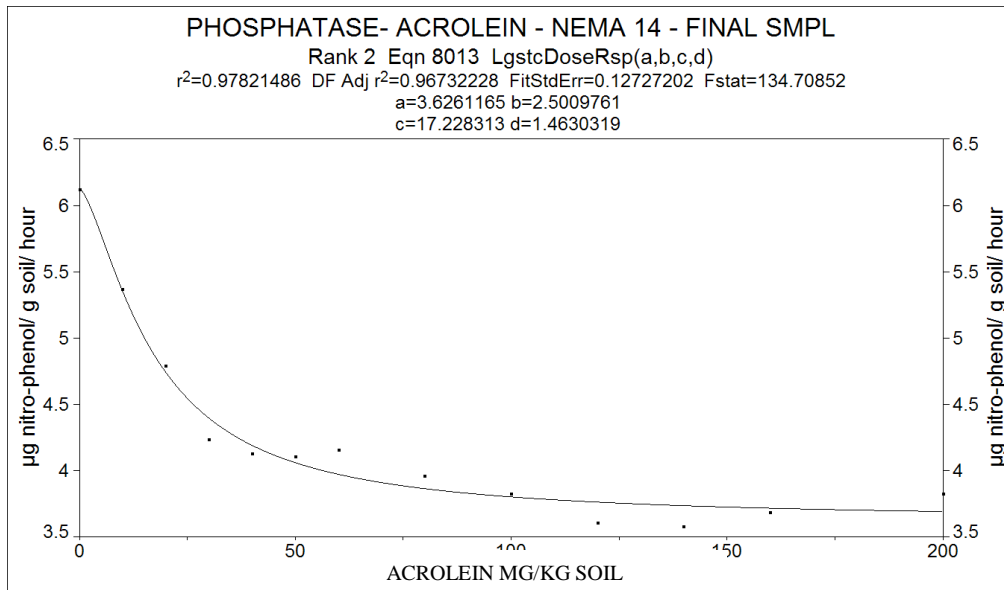


Figure 41I: Dosage response curve of soil phosphatase activity (Microbiology Experiment 7).

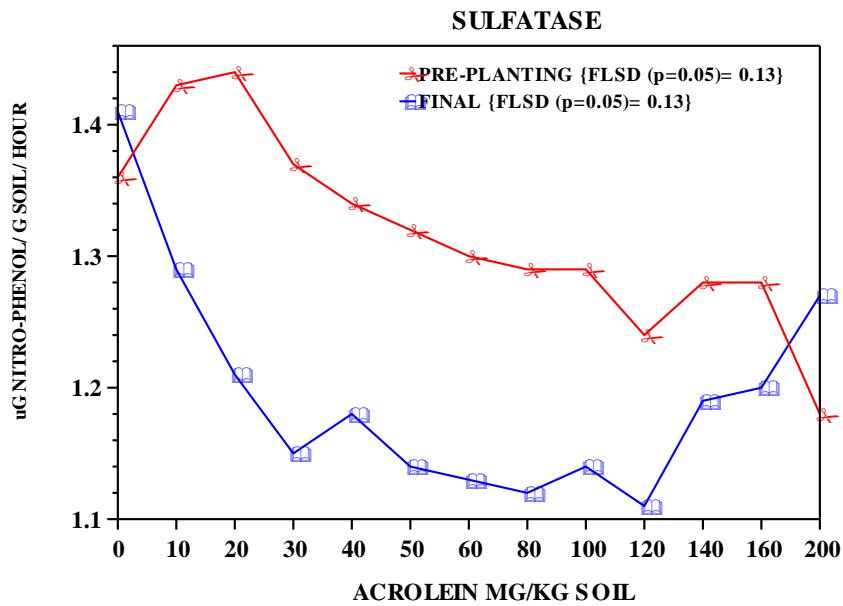


Figure 41J: Effects of drench-applied acrolein on soil sulfatase activity (Microbiology Experiment 7).

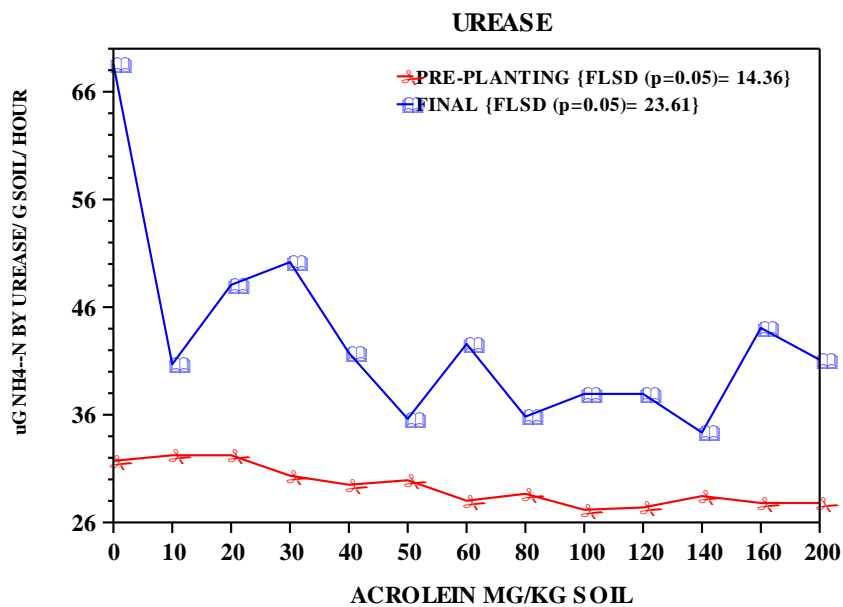


Figure 41K: Effects of drench-applied acrolein on soil urease activity (Microbiology Experiment 7).

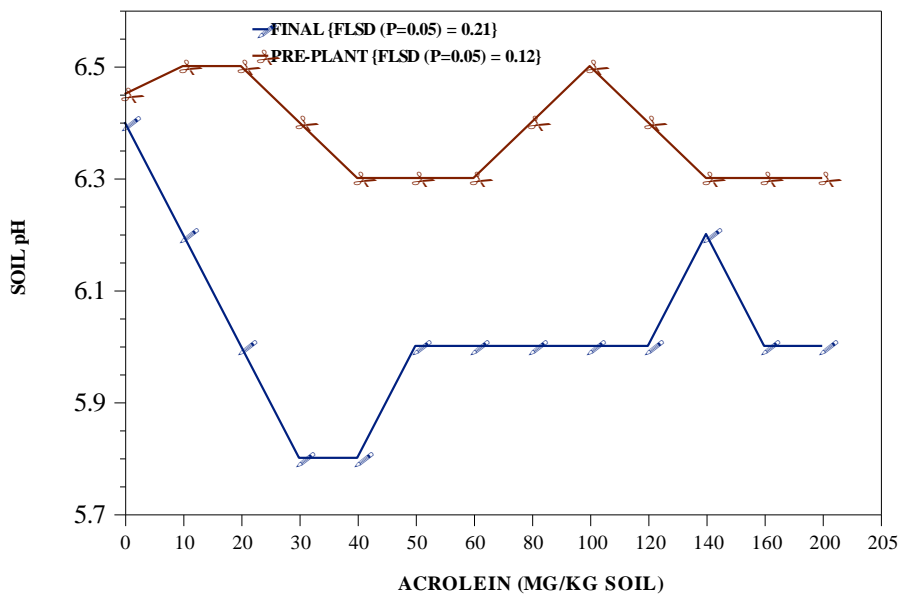


Figure 42A: Soil pH from pre-planting and final soil samples (Microbiology Experiment 8).

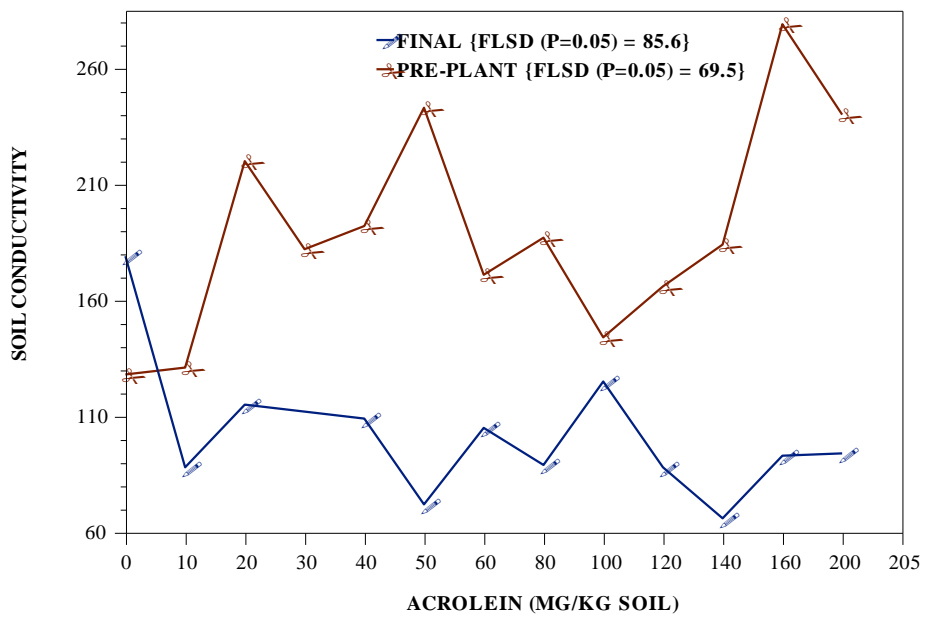


Figure 42B: Soil electrical conductivity from pre-planting and final soil samples (Microbiology Experiment 8).

V. DISSERTATION DISCUSSION

NEMATOCIDAL EFFICACY

Direct applications of acrolein to infested soil provided excellent control of the reniform nematode at rates of 75 - 100 mg/kg soil without drastically affecting microbivorous nematode populations. These rates of acrolein also resulted in healthier and larger plants suggesting that acrolein may be useful to disinfest agricultural fields prior to planting like methyl bromide. These acrolein rates are equivalent to 75 - 100 kg of acrolein per hectare (150 - 200 lbs/acre) which is less than the 150 - 225 kg of methyl bromide normally used to treat a hectare (300 - 450 lbs/acre), therefore, these rates of acrolein are within an acceptable range for agricultural use.

Unlike methyl bromide, acrolein is soluble in water and requires no emulsification to form aqueous solutions. The solubility of acrolein allows it also to be easily applied by drenching or through drip irrigation. Greenhouse studies indicate that drench applications of acrolein generally controlled root-knot, stubby-root, and spiral nematodes at 100 - 125 mg/kg soil without drastically affecting microbivorous nematode populations. Root-knot nematode was not controlled to the end of every experiment conducted, indicating that acrolein may be most effective if applied in combination with other nematicides or broad-spectrum pesticides.

The microplot experiment showed that acrolein can be an effective nematicide when applied as a drench. Root-knot and spiral nematodes were completely controlled by 100 kg/ha rate of acrolein 4 months after treatment while metam sodium at 92 L/ha stimulated populations of the nematodes. It is very significant that acrolein was more effective at 77 L/ha than a registered, broad-spectrum pesticide at like metam sodium at 92 L/ha.

Contrary to reports by McKenry *et al.* (1995) and Rahi and Rich (2003) acrolein may be an effective agricultural nematicide that can be applied by either direct application (shank injection), or in aqueous solution (drip and drench application). Effective nematicidal rates have not only resulted in better and healthier plants, but these rates of 100 - 125 mg acrolein/kg soil (200 - 250 lbs/acre) are within an acceptable range for use in agriculture.

HERBICIDAL EFFICACY

In general, higher rates of acrolein were required to control weeds than to control nematodes. Direct applications of acrolein controlled large crabgrass and pigweed at rates \leq 200 mg acrolein; however, 300 - 450 mg of acrolein was required to control yellow nutsedge. Low rates of acrolein (generally \leq 30 mg/kg soil) should be avoided as they can stimulate weeds including yellow nutsedge. Sedges are difficult to control, and when left uncontrolled they can be extremely detrimental in plastic-covered production systems like tomatoes, peppers, and cantaloupes. Drench applications, using aqueous solutions of acrolein, provided herbicidal efficacy at lower rates than required for direct applications. Most pre-emergent weeds were controlled with \leq 200 mg acrolein/kg soil,

while nutsedge required 200 - 300 mg of acrolein for control. Post-emergent control of weeds required lower rates of acrolein than needed for pre-emergent control.

When used to fumigate soil, acrolein provided excellent herbicidal efficacy, controlling most weeds at rates ≤ 200 mg/kg soil, and controlling yellow nutsedge with 200 - 250 mg. Aqueous solutions of acrolein used to fumigate soil reduced efficacy as the proportion of water in the solution increased. Results indicate that when using acrolein in aqueous solution to fumigate soil, the least amount of water may be the most effective. The soil moisture may have a significant impact on the efficacy of fumigating soils as acrolein treatments did not control weeds in saturated soils at the same rate as in soils at $\frac{1}{2}$ field capacity. Regardless of the method of application used, morningglory was the most sensitive to the compound of all the weeds studied, and yellow nutsedge the most tolerant with large crabgrass and sicklepod intermediate between those two species.

The herbicidal efficacy of acrolein was improved when it was applied in combination with propionic acid, butyric acid, EPTC, halosulfuron, *s*-metolachlor, and metam sodium. When applied in combination with any one of these six compounds, lower rates of both compounds were required to control weeds. These compounds served as complements to the herbicidal efficacy of acrolein and vice versa. Weeds, such as morningglory and large crabgrass that were not controlled well by the compounds alone, were controlled by the combinations with acrolein. Conversely, weeds not controlled well by acrolein alone, like nutsedge and sicklepod, were controlled by the combinations with the other compounds. Acrolein and metam sodium are not compatible for tank-mixing as the mixture lost herbicidal activity. Combinations with herbicides can provide

excellent herbicidal control in agricultural fields and significantly reduce the amount of acrolein that would be required if it was applied alone.

Acrolein is an effective aquatic herbicide (Anderson and Dechoretz, 1982;; Bentivegna *et al.*, 2005). Results of this research suggest it has potential for use in agriculture as a pre- and post-emergent herbicide. The effective herbicidal rates of acrolein are not only within an acceptable range for agricultural uses, but they generally result in improved plant growth and healthier plants. Combinations of acrolein with organic acids, certain herbicides, and other broad-spectrum pesticides can be used to increase herbicidal efficacy of acrolein at low rates. All effective herbicidal rates of acrolein caused no phytotoxicity to plants grown in the pots after treatment, indicating that these rates could be used to disinfest agricultural fields prior to planting.

EFFECT ON SOIL MICROBIOLOGY

Results from dipping caladium bulbs show that acrolein may be useful for disinfesting plant materials prior to planting. Disinfesting plant material and bulbs prior to planting is an effective means of disease prevention in several agricultural industries including ornamentals, potatoes, bananas, and various horticultural crops. Bioassay experiments suggest that acrolein is effective at controlling soil-borne seedling diseases at rates ≥ 40 mg acrolein.

Results from determinations of soil enzymatic activities show that as rates of acrolein are increased, enzymatic activities of the soil generally decreased. This is a common response to many broad-spectrum biocides used to sterilize soil. However, when microbial plating was conducted with acrolein-treated soils, it was apparent that acrolein was not acting as a biocide, rather it was a selective pesticide reducing some

groups of organisms while stimulating others. Numbers of soil bacteria decreased as acrolein rates were increased, while populations of actinomycetes and fungi increased as the rates of acrolein increased. Although the number of fungal colonies increased, the diversity of the fungal communities decreased to nearly one fungal genus: *Trichoderma*. The inclusion of glycerin in acrolein treatments increased incidence of *Trichoderma*. These are very positive results of acrolein treatments since *Trichoderma* spp. are known to compete with soil-borne plant pathogenic microorganisms, reduce disease incidence, and improve plant growth (Papavizas, 1985; Windham and Elad, 1986).

Acrolein applications in the range of 40 - 200 mg/kg soil, reduced seedling diseases while improving plant growth suggesting that the chemical may be useful as an agricultural pesticide. Acrolein did not create problematic microbiological voids in the soil, an important property for effective pest control and sustainable agricultural systems. Stimulation of actinomycetes and *Trichoderma* spp. populations suggest that acrolein treatment may actually improve the soil microflora while controlling plant deleterious organisms. This suggests that drench applications of acrolein could result in soils that are less conducive for soil-borne seedling diseases. Since no phytotoxicity was observed, and plant growth parameters measured were all improved, acrolein may be useful for disinfecting agricultural soils prior to planting.

ACROLEIN AS AN ALTERNATIVE TO METHYL BROMIDE

Acrolein provided effective control of plant parasitic nematodes, weeds, and soil-borne diseases while not harming all beneficial soil organisms. Effective rates of acrolein also result in improved and healthier plants. All effective rates are within an acceptable range for applications in agriculture, and when combined with other compounds, less

acrolein is required for efficacy. This is also true with methyl bromide as it is typically applied in combination with chloropicrin. Acrolein can be applied with a methyl bromide injection apparatus with only minor modifications. Unlike methyl bromide, acrolein is soluble in water allowing it to be applied in aqueous solutions without the need for emulsification. In contrast to methyl bromide, acrolein has a strong irritating odor and is a lachrymatory compound. This is an advantage to alert humans of the presence of acrolein. Acrolein has U.S. registration as an aquatic herbicide which may help facilitate extensions of registration for soil applications. Applications of acrolein improve the soil microflora and may create a soil environment suppressive to plant diseases. Applications of acrolein have also shown to improve plant health and stimulate growth responses. Acrolein has proven its potential to control agricultural pests and improve crops. Acrolein warrants further development as an alternative to methyl bromide and possibly U.S. registration as a soil-applied, broad-spectrum pesticide for agricultural use.

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APPENDIX

I. METHODS

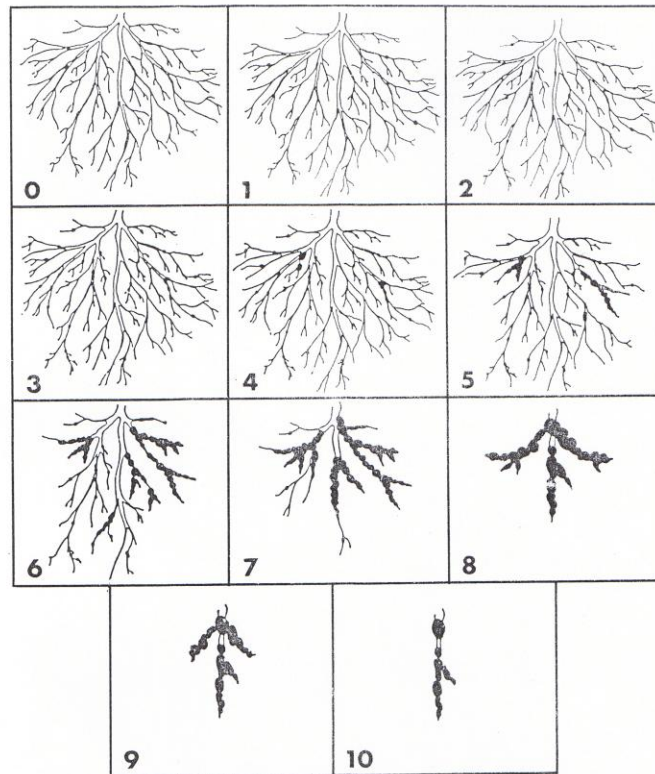
SOIL SAMPLING

For the greenhouse trials, the entire pot (1 kg) is poured into a bag, shaken to evenly mix, and a 100 cm³ sample is taken. Soil samples in the microplots and field are taken using a 2.5 cm diameter soil probe around the root zones of the crops planted. Random samplings of the root zones within a treatment are collected in a bag. The bagged samples from the field are taken to the lab where they are mixed evenly and a 100 cm³ subsample is taken.

NEMATODE ANALYSIS

Nematodes are extracted from soil samples using an incubation technique, referred to as the “salad bowl” method (Rodríguez-Kábana and Pope, 1981). Soil samples are deposited in a tissue paper placed on a large-mesh container that is placed in a salad bowl with enough water to submerge the sample. Root samples are processed the same except tissue paper is not required. Samples are incubated at room temperature for 3 days, when the screens with samples are then removed and the contents of the salad bowl are poured through a 400-mesh screen. Nematodes are captured and concentrated in the 400-mesh screen and are then transferred to a petri dish for identification and counting. This method is quite common and is very practical for the needs of the study.

ROOT-KNOT RATING SCALE (ZECK,1971)



Rating scheme for evaluation of root-knot infestation
(Zeck)

Scale: 0 = no infestation, 10 = maximum infestation

SOIL UREASE ACTIVITY DETERMINATION (by micro-diffusion):

Enzymatic Incubation Procedure:

- 1) Label and arrange 30 ml solo cups for samples to be analyzed.
- 2) Place 2 g of soil sample into appropriate cups.

- 3) Add 2 ml of 3% urea (substrate) to each cup and incubate a 37°C for 3 - 5 hours (more incubation time is needed for samples with low activity). Be sure to include water controls in which no urea is added.
- 4) Add 10 ml of 10% NaCl (pH 2.1) to halt reaction and then centrifuge for 20 - 30 minutes at 3000 rpm.
- 5) Label and arrange micro-diffusion chambers for samples and procedural controls. (Include chambers with no sample in the cup to serve as procedural controls.)
- 6) In each chamber place (2) 15 ml soufflé cups (mark at least one so the “trap” and the sample do not become confused with one another).
- 7) Into one soufflé cup place 2 ml supernatant from the centrifuged samples
- 8) Into the other soufflé cup deliver 5 ml of 0.1 N HCl to serve as the “trap”.
- 9) Into the sample cup deliver 5 ml of 20% Na₂CO₃ and close/cap chamber immediately.
- 10) Allow chambers to incubate over-night. (This may be much more time than needed, but this has not been explored.)
- 11) Take 1 to 5 ml from the HCl trap for color determination.

Color Determination Procedure:

- 1) In 30 ml plastic cups, place 1 ml supernatant from the samples + 5 ml solution A + 2 ml solution B.
- 2) Wait 30 - 45 minutes for color development (room temperature). Yellow color indicates little or no enzymatic activity, the greener the color that develops indicates more urease activity.

3) Determine absorbance values in a spectrophotometer at 690 nm wavelength. (Milton Roy Company Spectronic 601 used for determinations made for this dissertation).

☼ Important Note – Ammonium nitrogen must be subtracted from the urease products ($\text{NH}_4\text{-N}$) for accurate activity determination.

- To determine existing $\text{NH}_4\text{-N}$ in soil, process the samples again substituting 2 ml water instead of the urea solution and add 10 ml of (pH 2.5) NaCl 10%.

- Wait 1 - 1.5 hours at room temperature to ensure NH_4 replacement is complete.

- Control- 4 ml water + 10 ml NaCl 10%. Then take 1 ml of this solution and add 5 ml solution A + 2 ml solution B (used to zero).

→Standard Curve (using NH_4Cl):

- 1) Dissolve 3.82 g of NH_4Cl in deionized water; using a volumetric flask make up final volume 1000 ml with deionized water. Solution (containing 1000 μg $\text{NH}_4\text{-N}$ /ml) is stable for several months in a dark bottle stored at 4°C with a drop of chloroform.
- 2) Calibration curve is developed using 0, 2, 4, 8, 10, 14, 18, and 20 μg $\text{NH}_4\text{-N}$ /ml in deionized water.
- 3) In a plastic cup, place 1 ml of each dilution + 5 ml of solution A + 2 ml of solution B.
- 4) Control- (for zeroing) use 1 ml of water + 5 ml of solution A + 2 ml of solution B.

5) Wait 30 minutes at room temperature, then determine absorbance with a spectrophotometer set at 690 nm wavelength.

→Calculation of Urease Activity:

- Transform OD values to $\text{NH}_4\text{-N}$ concentrations from urease activity using the appropriate curve (formula $y_1 = a + bx_1$).

- Use the same procedure of transformation to determine values of existing $\text{NH}_4\text{-N}$ (no urea substrate) using the appropriate curve (formula $y_2 = a + bx_2$).

- Calculate $z_1 = (y_1)$ (dilution used: 10 ml NaCl + 2 ml urea) (trap dilution; 5ml) / (soil sample weight 2 g) (supernatant in diffusion; 2ml)

therefore $z_1 = (y_1)(6) = \text{NH}_4\text{-N total (product of urease activity + existing NH}_4\text{-N)}$.

- Calculate $z_2 = (y_2)$ (12 ml water) / (2 g) therefore $z_2 = (y_2)(6) = \text{existing NH}_4\text{-N (already present in the soil)}$.

- Calculate $\mu\text{g NH}_4\text{-N (g soil)}^{-1} (\text{h})^{-1}$ by urease activity = $(z_1 - z_2) / (\text{incubation time 3 h})$.

→Solution A Preparation (daily):

- First dissolve 17 g of Na-salicylate in distilled water and then slowly add 120 mg Na-nitroprusside while stirring.

- Bring final volume to 100 ml with distilled water. This solution is stable for months in a dark bottle at room temperature.

- Then mix 1:1:1 solution of Na-salicylate solution: 20% (w/v) sodium carbonate solution: deionized water.

☼ 5 ml of solution A are needed per sample.

→Solution B Preparation (daily):

- Dissolve 0.1 g of dichloro-isocyanuric acid sodium salt in 100 ml distilled water to have a 0.1% solution (w/v).

☀ Solution B can be used between 30 minutes to 8 hours after preparation.

ENZYMATIC DETERMINATION WITH NITROPHENOL BASED SUBSTRATES:
CHITOBIASE, α -GLUCOSIDASE, β -GLUCOSIDASE, α -GALACTOSIDASE, β -
GALACTOSIDASE, PHOSPHOTASE, AND SULFATASE.

- 1) Weigh 2 g of the air-dried samples into a 30 ml plastic cups.
- 2) Add 2 ml of substrate (1 ml per g soil).
- 3) Controls- (1) 4 ml water, (2) 2 ml water + 2 ml substrate, and (3) 2 g sterile soil + 2 ml substrate.
- 4) Shake cups gently and seal between 2 trays.
- 5) Incubate at 37°C for 3 - 15 hours depending on expected activity. (5 hours for phosphatase, sulfatase, and β –glucosidase; 15 hours for the others worked well in this study).
- 6) After incubation, stop the reaction by adding 10 ml of 95% ethyl alcohol and shaking gently. Include controls in this step to maintain the same dilution factor for all samples.
- 7) Allow 10 - 15 minutes for particles to settle, then pour all liquid into centrifuge tubes. Centrifuge at approximately 3000 rpm, 20 minutes for soil, 10 minutes for sand.

- 8) Prepare samples for colorimetric analysis by placing 10 ml water + 2 ml supernatant in 30 ml plastic cups (If high activity is expected use 1 ml supernatant).
- 9) Then add 1 ml 0.2 N NaOH for color development.
- 10) Shake cups gently for a few seconds.
- 11) Read the samples in a spectrophotometer at 420 nm wavelength and record absorbance values. (Milton Roy Company Spectronic 601 used for determinations made for this dissertation).

- For proper baseline, zero the instrument with pure water first, then with control 1, then with control 2, finally zero the instrument with control 3 before analyzing samples. Re-zero with control 3 after every 10 - 15 samples to ensure accuracy.

→Reaction is Colorimetric:

p-nitrophenol-based substrate → p-nitrophenol (yellow color with NaOH) + products

→Substrate preparation:

- Substrates are dissolved in deionized water to make final concentrations of 1 mg/ml.

- Substrates used: chitobiase (p-nitrophenyl-N-acetyl-β-D-glucosaminide), β-glucosidase (p-nitrophenyl-β-D-glucopyranoside), phosphatase (p-nitrophenyl phosphate disodium•6H₂O), and sulfatase (4-nitrophenyl sulfate, potassium salt, 99%).

→Standard Curve using p-Nitrophenol:

- 1) Prepare a stock solution of 1 mg p-nitrophenol/ml ethanol solution by placing 0.1 g p-nitrophenol in a volumetric flask and bringing final volume to 100 ml

using 95% ethanol. This can be hermetically sealed in a dark bottle for storage at 4°C for several months.

- 2) Dilutions of stock solution are made with 95% ethanol and will include: 0, 1, 2, 4, 8, 10, 20, and 30 µg/ml p-nitrophenol. Vortex the dilutions.
- 3) Zero spectrophotometer using the 0 µg/ml (420 nm).
- 4) Mix 2 ml of each dilution + 10 ml of water + 1 ml 0.2 N NaOH and vortex again.
- 5) Record absorbance values read from a spectrophotometer set at 420 nm.

→Calculation of nitrophenyl based Substrate Enzymatic Activity:

- Transform absorbance values to p-nitrophenol concentrations from enzymatic activity using appropriate standard curve ($y = a + bx$).
- Calculate $z = (y) (\text{dilution used: } 10 \text{ ml ethanol} + 2 \text{ ml substrate}) / (\text{soil weight g} (\text{time h}))$ therefore $z = (y \times 12 \text{ ml}) / (2 \text{ g}) (\text{h}) = \mu\text{g p-nitrophenol (g soil)}^{-1} (\text{h})^{-1}$.

SOIL CATALASE ACTIVITY DETERMINATION

- 1) Weigh 5 g of air dried soil samples in 30 ml plastic cups.
- 2) Add 5 ml deionized water + 5 ml H₂O₂ 0.3% (v/v).
- 3) Cover the cups and incubate at 28°C for 30 minutes (normal soil) to 1 hour (sandy soil). Controls- (1) 15 ml water, (2) 10 ml water + 5 ml H₂O₂ 0.3%, and (3) 5 g sterile soil + 5 ml water + 5 ml H₂O₂ 0.3%.
- 4) Halt reaction (and acidify) by adding 5 ml 3N H₂SO₄ and shaking well.

- 5) Take a 5 ml aliquot of the clear supernatant for titration with 0.02 N KMnO_4 .
- 6) Add two drops of 1% MnSO_4 just before titration to speed the reaction.

- Amount of 0.02 N KMnO_4 for titration should be about only one drop 0.02 N KMnO_4 for control 1, about 14 - 15 ml for controls 2 and 3, and 2 - 15+ for the samples.

→Standardize 0.02 N KMnO_4 –

- 1) Mix 5 ml deionized water + 5 ml 0.05 M Na Oxalate + 5 ml 3N H_2SO_4 .
- 2) Take a 5 ml aliquot and place in 30 ml plastic cup and add one drop 1% MnSO_4 .
- 3) Titrate with 0.02 N KMnO_4 until color change is noticed.
- 4) Repeat titration at least 3 times to ensure accuracy.
- 5) Calculate normality with:

$$(\text{ml } \text{KMnO}_4) (\text{N}) = \frac{(\text{ml aliquot}) (\text{Normality of oxalate})}{(\text{proportion used in the aliquot})}$$

→Calculation of Catalase Activity:

- 1) Subtract control 3 value from samples to obtain enzymatically decomposed H_2O_2 from the samples.
- 2) Calculate catalase activity as milli-equivalents of decomposed H_2O_2 using formula:

$$\text{meq } \text{H}_2\text{O}_2 = (\text{difference of ml N } \text{KMnO}_4) \times (\text{Normality of } \text{KMnO}_4) \times (1/\text{time}) \times (1/\text{g soil}) \times (\text{dilution factor}).$$

☼Time and temperature of incubation must be included with catalase activity data as the reaction is dependant on time and temperature.

SOIL pH AND CODUCTIVITY

pH:

- 1) Weigh 5 g of soil samples to be analyzed in 30 ml plastic cups.
- 2) Add 10 ml of demineralized water to each cup.
- 3) Shake by hand or with a mechanical shaker.
- 4) Stir slurry with plastic coffee stirrer just before determining pH with an appropriate probe.
- 5) Sample reading : X.XY when Y reaches 8, round up.

CONDUCTIVITY:

- 1) Pour supernatant from pH cups into polypropylene centrifuge tubes.
- 2) Centrifuge at $\frac{3}{4}$ speed for 20 - 25 minutes (or until clear).
- 3) Determine soil conductivity with an appropriate instrument.

SOIL MICROBIOLOGY

PREPARING SOIL SOLUTIONS FOR PLATING:

- 1) A 50 cm³ sample is taken from a well mixed soil sample.
- 2) 10 g fresh soil is weighed into separate containers.
- 3) Prepare agar-based microbial growing media for fungi, bacteria, and actinomycetes.
- 4) Prepare 250 ml Erlenmeyer flasks for the test by: labeling according to test, filling with 98 ml demineralized water, covering with Whatman No. 1 filter paper, sealing with a rubber band, capping with aluminum cap or aluminum foil, and steaming in an autoclave for 20 - 30 minutes.

- 5) Prepare 500 ml Erlenmeyer flasks for the test by: labeling according to test, filling with 340 ml demineralized water, covering with Whatman No. 1 filter paper, sealing with a rubber band, capping with aluminum cap or aluminum foil, and steaming in an autoclave for 20 - 30 minutes.
- 6) Prepare 5 or 10 ml pipettes and precision glass droppers with long attenuate tips by placing in autoclavable container or aluminum foil and steaming in an autoclave for 20 - 30 minutes.
- 7) Prepare aluminum weigh boats by numbering with a nail or sharp object as many as needed for the test. Carefully weigh and record the weight of each boat.
- 8) 10 g soil samples are added to appropriately labeled 250 ml Erlenmeyer flasks containing 98 ml of demineralized water approximately ten minutes before use.
- 9) 250 ml flasks of soil solutions are placed on a magnetic stirrer with a 1.5 inch stir bar in the flask. Using the same setting for all samples is important. Allow sample to stir for at least 30 seconds before taking out any drops of suspension.
- 10) Using a precision glass dropper with a long attenuate tip and soft latex bulb, take sub-samples one at a time from the same location in the vortex of the stirring soil suspension. Take one sub-sample or “draw” and carefully deliver one drop into the prepared aluminum weigh boat.
- 11) Repeat 19 more times taking care to deliver drops of soil suspension that are the same size.

- 12) Add one sub-sample drop into a sterile petri plate, near the edge is preferred.
- 13) Repeat this with at least four more sterile petri plates for the one soil sample.
- 14) Pour an agar-based medium prepared for the selection of fungi over the droplets in each of the plates until the medium covers approximately $\frac{3}{4}$ - $\frac{3}{5}$ of the plate.
- 15) Stack the plates and gently swirl to distribute the soil suspension droplet and for the medium to cover the bottom of the plate. Take care to distribute the droplet and not to swirl media out of the plate.
- 16) Take 5 ml from the soil suspension while it is still stirring and deliver it into the appropriately labeled 500 ml Erlenmeyer flasks containing 340 ml demineralized water.
- 17) Place these more dilute soil suspensions one at a time on the magnetic stirrer and allow to mix for at least 30 seconds before taking any sub-samples.
- 18) Using a precision glass dropper with a long attenuate tip and soft latex bulb, take sub-samples one at a time from the same location in the vortex of the stirring soil suspension. Take one sub-sample or “draw” and carefully deliver one drop into a sterile petri plate near the edge.
- 19) Repeat this with at least four more sterile petri plates for the one soil sample.
- 20) Pour an agar-based medium prepared for the selection of bacteria over the droplets in each of the plates until the medium covers approximately $\frac{3}{5}$ of the plate.

- 21) Stack the plates and gently swirl to distribute the soil suspension droplet and for the medium to cover the bottom of the plate. Take care to distribute the droplet and not to swirl media out of the plate.
- 22) Allow plates to incubate and then make counts and identifications.
- 23) Dry aluminum weigh boats in an oven with a temperature of 102°C – 107°C overnight.
- 24) Weigh dry boats with soil carefully and record the data. Subtract the weight of the boat (initial weight) from the weight of the boats plus the soil (final dried weight) to calculate the amount of soil per drop. This is used to determine the total colony forming units (CFUs) per gram of soil.

FOR ACTINOMYCETES PLATING

→ Repeat steps 1 through 15 of the same procedure, only use soil samples weighing 2 grams instead of 10 grams.

MICROBIOLOGICAL CULTURE MEDIAS:

FOR FUNGI:

OHIO MEDIUM: (Schmitthenner and Williams, 1958) from Methods for the Research on the Ecology of Soil-Borne Plant Pathogens by Johnson and Curl.

- 1) Using a 1000 ml Erlenmeyer flask, heat one liter of tap water in microwave for seven minutes.
- 2) Place flask on a magnetic stirrer, add a 2 inch magnetic stir bar into the flask, and create a large vortex.

3) Weigh out and add to the vortex:

a) Agar.....	20.0 g
b) Glucose.....	5.0g
c) Yeast Extract.....	2.0g
d) NaNO ₃	1.0g
e) MgSO ₄ •7H ₂ O.....	0.5g
f) KH ₂ PO ₄	1.0g
g) Oxgall.....	1.0g
h) Sodium Propionate (Propionic acid, sodium salt).....	1.0g

4) After these ingredients have melted pour into autoclavable flasks and be sure to allow enough room so that the agar does not boil over in the autoclave.

5) Cap media flasks with Whatman No. 1 filter paper and close with a rubber band.

6) Cap off with aluminum foil and label with autoclave-safe tape and possibly autoclave indicator tape.

7) Place in preheated autoclave and sterilize for 20 minutes or more, depending on how much is being sterilized.

8) After sterilization, allow to cool and place in a cabinet or cover with a cloth until needed for use.

9) Melt agar for use by placing in a pre-heated autoclave and steam for however long needed to melt.

10) Place in a preheated water bath set just at temperature that is cool enough to handle.

11) Prepare a 10 mg/ml solution of Chloromycetin (Chloramphenicol) and add 50.0 mg to each 1000 ml of Ohio medium.

12) Prepare a 10 mg/ml solution of Streptomycin sulfate and add 50.0 mg to each 1000 ml of Ohio medium.

→Medium is now ready for use.

FOR BACTERIA

THORNTON'S MEDIUM: (Thornton, 1922) from Methods for the Research on the Ecology of Soil-Borne Plant Pathogens by Johnson and Curl.

1) Using a 1000 ml Erlenmeyer flask, heat one liter of tap water in microwave for seven minutes.

2) Place flask on a magnetic stirrer, add a 2 inch magnetic stir bar into the flask, and create a large vortex.

3) Weigh out and add to the vortex:

a) Agar.....	15.0 g
b) K ₂ HPO ₄	1.0g
c) MgSO ₄ •7H ₂ O.....	0.2g
d) CaCl ₂	0.1g
e) NaCl.....	0.1g
f) FeCl ₃	trace
g) KNO ₃	0.5g
h) Asparagine.....	0.5g
i) Mannitol.....	1.0g

- 4) After these ingredients have melted pour into autoclavable flasks and be sure to allow enough room so that the agar does not boil over in the autoclave.
 - 5) Cap media flasks with Whatman No. 1 filter paper and close with a rubber band.
 - 6) Cap off with aluminum foil and label with autoclave-safe tape and possibly autoclave indicator tape.
 - 7) Place in preheated autoclave and sterilize for 20 minutes or more, depending on how much is being sterilized.
 - 8) After sterilization, allow to cool and place in a cabinet or cover with a cloth until needed for use.
 - 9) Melt agar for use by placing in a pre-heated autoclave and steam for however long needed to melt.
 - 10) Place in a preheated water bath set just at temperature that is cool enough to handle.
- Medium is now ready for use.

FOR ACTINOMYCETES

BENEDICT AGAR (Porter *et al.*, 1960) from: Methods for the Research on the Ecology of Soil-Borne Plant Pathogens by Johnson and Curl.

- 1) Using a 1000 ml Erlenmeyer flask, heat one liter of tap water in microwave for seven minutes.
- 2) Place flask on a magnetic stirrer, add a 2 inch magnetic stir bar into the flask, and create a large vortex.

3) Weigh out and add to the vortex:

- a) Agar.....20.0 g
- b) Glycerol.....20.0g
- c) L-arginine.....2.5g
- d) NaCl.....0.1g
- e) FeSO₄•7H₂O.....0.1g
- f) CaCO₃.....0.1g
- g) MgSO₄•7H₂O.....0.1g

4) After these ingredients have melted pour into autoclavable flasks and be sure to allow enough room so that the agar does not boil over in the autoclave.

5) Cap media flasks with Whatman No. 1 filter paper and close with a rubber band.

6) Cap off with aluminum foil and label with autoclave-safe tape and possibly autoclave indicator tape.

7) Place in preheated autoclave and sterilize for 20 minutes or more, depending on how much is being sterilized.

8) After sterilization, allow to cool and place in a cabinet or cover with a cloth until needed for use.

9) Melt agar for use by placing in a pre-heated autoclave and steam for however long needed to melt.

10) Place in a preheated water bath set just at temperature that is cool enough to handle.

11) Prepare a 10 mg/ml solution of Pimaricin and add 50.0 mg to each 1000 ml of Benedict medium.

→Medium is now ready for use.