The fate of bifenthrin and fipronil in pine bark nursery media

Russell Stanley Harris, III  
*Louisiana State University and Agricultural and Mechanical College, rharr21@lsu.edu*

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THE FATE OF BIFENTHRIN AND FIPRONIL
IN PINE BARK NURSERY MEDIA

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Horticulture

by
Russell Stanley Harris, III
B.S., Louisiana State University, 1997
August 2004
La vie est amère,
mais les chiens
sont heureux.

-A favorite saying of my grandfather,

Russell Stanley Harris, Sr.
DEDICATION

This is dedicated to all the people who have contributed to my life-long love of and passion for horticulture:

To love of my life, Carol L. Miller, for providing me with the love and the support, during my pursuit of this degree, that only a best friend and love can provide.

To my mother and father, Earlita F. Harris and Russell S. Harris, Jr., for your unconditional love, support, understanding, and encouragement. You have guided me through life and provided me with the skills necessary to pursue my dreams and make them come true. If it weren't for you, I would not be here today.

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ABSTRACT

In recent years, concerns over the adverse effects of pesticides on human health and the environment have led to the development of stricter pesticide regulations and the outright banning of many pesticides in the U.S. Bifenthrin and fipronil are important pesticides used in the nursery industry for the control of imported fire ants (*Solenopsis invicta* Buren, *S. richteri* Forel, and their hybrids) to meet the requirements of the Imported Fire Ant Quarantine. Although bifenthrin and fipronil have been in use for many years and their fate in natural soils has been studied extensively, they were recently labeled for use in the nursery industry. Nurseries typically use highly-organic media ("soilless" media) and little is known about the fate of these pesticides when used with these media.

Our research measured the influence of irrigation frequency and time on the degradation of bifenthrin and fipronil in a nursery medium composed of 90% pine bark and 10% mason sand. Media samples and media leachate samples were collected over a period of 180 days. Levels of bifenthrin, fipronil, and fipronil metabolites (MB45950, MB46136, MB46513) were measured using gas chromatography with electron capture detection (GC/ECD) and gas chromatography secondary ion mass spectrometry (GCMS/SIMS).

Bifenthrin levels in the nursery potting media initially dropped and then remained constant throughout the study. Fipronil levels in nursery potting media decreased over time. Levels of the fipronil metabolite MB45950 in potting media
fluctuated over time, while the levels of this metabolite increased over time in media leachates. Levels of the fipronil metabolite MB46136 in potting medium increased slightly and then remained constant over time, while levels of this metabolite increased over time in medium leachates. The metabolite of fipronil, MB46513, was not detected during this study in potting medium or medium leachates. In this study, levels of bifenthrin and fipronil detected in potting medium leachates were higher than their known solubilities in water. It is likely that these increased levels are due to the high levels of organic acids, phenols, terpenes, resins and natural solvents found in pine bark as well as minute organic particles suspended in the leachates. It is also possible that media and leachate temperatures and the pH of irrigation water affected the fate of these chemicals.
1.1 Introduction

Horticulture, especially the production of nursery stock and ornamentals, is an important part of the economy in the state of Louisiana (Anonymous, 2004). In recent years this sector of the economy has grown rapidly (Hinson et al., 2003). However, restrictions placed on the trade of ornamentals and nursery stock between areas with and without infestations of imported fire ants by the Federal Imported Fire Ant Quarantine Program, (Callcott, 2003) have resulted in economic losses (Anonymous, 2004a). As a result, extensive research has been done in an effort to control these ants (Williams et al., 2001).

There are several methods utilized by the nursery industry that use pesticides to control imported fire ants in an effort to ease trade restrictions between areas with and without infestations. Granular formulations of either bifenthrin or fipronil incorporated into potting media are two of the methods used (Callcott, 2003). A review of current literature has shown that little is known about the fate of these two pesticides in the highly organic potting media used in the production of containerized nursery stock. It was also found that previous research has focused on the fate of bifenthrin and fipronil in natural mineral soils which have very different chemical and physical properties than nursery potting media (Bunt, 1986). Therefore, research into the fate of these two chemicals in nursery potting medium is needed. The focus of this thesis is the fate of bifenthrin and fipronil in nursery potting media. The objectives of this research are to quantify and predict the presence of bifenthrin, fipronil, and fipronil metabolites in pine bark nursery media and media leachate and to measure the affect of two irrigation
regimes (1 X and 3 X) on the quantities of bifenthrin, fipronil, and fipronil metabolites in potting media and media leachate.

1.2 Literature Review

1.2.1 Louisiana’s Green Industry

The nursery and landscape industry, also known as the “green industry,” is an important component of Louisiana’s economy. The green industry can be defined as “the production, sale and maintenance of ornamental plants and related products and includes the golf course industry, farm production of plant materials, and the service industries that provide design, installation and/or maintenance of home lawns and gardens, public and commercial grounds” (Hinson et al., 2003). In 1995, the green industry contributed $1.3 billion in gross sales, $485.97 million in total personal income, $848.35 million in gross state product, and 26,277 jobs to the Louisiana economy. In recent years, the Louisiana green industry grew rapidly and in 2001 its economic contributions were reported to be $2.2 billion in gross sales, $1.15 billion in total personal income, $1.69 billion in gross state product, and 56,686 jobs (Hinson et al., 2003). The 2004 gross farm income for nursery stock and ornamentals was reported to be $106,973,500.00 and value added was $54,556,485.00 for Louisiana (Anonymous, 2004).

Unfortunately, Louisiana and many other states suffer tremendous monetary losses directly related to infestations of imported fire ants. The Center for Bioenvironmental Research at Tulane and Xavier Universities estimates $2 billion in national annual economic losses due to fire ants (Anonymous, 2004a). Imported Fire Ants (IFA) currently infest more than 320 million acres in Alabama, Arkansas, California, Florida,
Georgia, Louisiana, Mississippi, New Mexico, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, and Puerto Rico (Callcott, 2003).

1.2.2 Fire Ant Classification

Modern insects first appeared on Earth during the Permian Period [290 to 248 million years ago (Anonymous, 2004b)] during the Paleozoic era (Borror et al., 1989). Ants are social insects that have lived on Earth for more than 80 million years. There are currently 292 living genera of ants with approximately 8,800 known species and, today, ants are found everywhere in the world with the exceptions of Antarctica, Iceland, Greenland, parts of Polynesia, and a few remote islands in the Atlantic and Pacific oceans (Hölldobler et al., 1990). Ants belong to the phylum Arthropoda, the subphylum Atelocerata, class Hexapoda, and the order Hymenoptera (Borror et al., 1989). In addition to ants Hymenoptera contains sawflies, horntails, parasitic wasps, wasps, and bees. Hymenoptera is separated into two suborders, Symphyta and Apocrita and ants are part of Apocrita. Apocrita is divided into several superfamilies. The superfamily Formicoidea contains only one family Formicidae to which ants belong. Formicidae is divided into several subfamilies and fire ants belong to the subfamily Myrmicinae and comprise the genus Solenopsis.

There are eighteen to twenty species of fire ants in the genus Solenopsis, all of which are native to the New World (Taber, 2000). Six true species of fire ants live in the United States. Three of the six species living in the United States are native and are Solenopsis amblychila Wheeler (no common name), S. aurea Wheeler (the desert or golden fire ant), and S. xyloni McCook (the southern fire ant). The three non-native species are S. geminata Fabricius (the tropical fire ant), S. invicta Buren (the red
imported fire ant (RIFA); *S. savissima* var. *wagneri*; *S. wagneri* Santschi), and *S. richteri* Forel (the black imported fire ant (BIFA); *S. saevissima richteri*). In addition, hybrids between *S. invicta* and *S. richteri* exist (Taber, 2000). The focus of this study will be *S. invicta*, *S. richteri* and their hybrids, and will be collectively referred to as Imported Fire Ants (IFA).

1.2.3 Fire Ant Ecology

Ants are valuable in biological systems because they process more soil than earthworms (Hölldobler *et al.*, 1990). These insects are major predators of other insects and small invertebrates, recycle nutrients from dead animals and plants, serve as a food source for other animals, and are responsible for the dispersal of significant amounts of seeds. Germination and growth of these seeds, accounts for up to 40% of the biomass in some areas. In the U.S., fire ants play an important role in biological, agricultural, and urban systems as both beneficial insects and destructive and harmful pests (Hölldobler *et al.*, 1990). Imported fire ants are beneficial to the U.S. sugarcane industry as a biological control agent of sugarcane borer (Reagan, 2001); however they are considered an invasive species to the U.S. (Anonymous, 2004c).

Historical records indicate IFA arrived in the United States independently. The first species to arrive, *S. richteri*, is native to Argentina and was first reported on the Gulf Coast by Henry Peter Löding in 1918 near the Port of Mobile, Ala. (Taber, 2000). The second arrival, *S. invicta*, is native to Brazil and also arrived through the port of Mobile, Ala. in the 1930s. Both species are believed to have been stowaways on cargo ships, possibly infesting ballast, dunnage and/or shipments of agricultural goods. *Solenopsis richteri* increased its range rapidly until the arrival of *S. invicta*, which out-
competed it and limited its range. In addition, where their ranges have overlapped, hybridization has occurred resulting in fertile offspring. These hybrids now occupy a larger range than *S. geminata* and have formed a buffer zone between the two species, eliminating their direct competition with one another.

Since their arrival, IFA have spread, with the aid of human activities, from their ports of entry across the Southern U.S., into California, up the East Coast, and to Puerto Rico (Fig. 1.1). Isolated infestations have been reported in other areas, but have been eliminated. Today, IFA infest more than 320 million acres and is expected to increase its range until stopped by low winter temperatures. However, some experts speculate that IFA will spread into cold climates as an annual pest that takes refuge during winter months in warm niches located in agricultural, industrial, and residential areas (Williams *et al.*, 2001).

### 1.2.4 Federal Fire Ant Quarantine

In an effort to control the spread of imported fire ants (*Solenopsis invicta* Buren, *S. richteri* Forel, and their hybrids), the U.S. Dept. of Agriculture (USDA) enacted the Federal Imported Fire Ant Quarantine Program (IFA quarantine) on 6 May 1958. The USDA Animal and Plant Health Inspection Service (APHIS) implemented the IFA quarantine (Callcott, 2003) in cooperation with IFA infested states. These organizations assess the effectiveness of quarantine treatments, and participate in research and development with industrial, federal, and state agencies interested in finding new insecticides and biological control agents (Anonymous, 2004c). The IFA quarantine (Title 7, Code of Federal Regulations, Part 301.81) regulates and requires that the following be issued a certificate or permit before they are shipped outside of
quarantined areas: imported fire ant queens and reproducing colonies of imported fire ants; soil, separately or with other items, except soil samples shipped to approved laboratories; however, potting soil is exempt if commercially prepared, packaged, and shipped in original container; plants with roots and soil attached, except house plants maintained indoors and not for sale; grass sod; baled hay and straw that has been stored in contact with soil; used soil-moving equipment; and any other products, articles, or means of conveyance when it is determined by an inspector that they may facilitate the spread of the IFA (Callcott, 2003).

Fig. 1.1. Imported fire ant quarantine map of the U.S. Shaded areas indicate restrictions on the movement of articles regulated by the Federal Fire Ant Quarantine to unshaded areas. Movements of regulated articles within shaded areas are unrestricted (Callcott, 2003).

People in possession of articles regulated under the Federal Imported Fire Ant Quarantine Program will not be issued a certificate or permit allowing the items to be shipped outside of the quarantined areas unless they are IFA free. The presence of one fire ant in a regulated item is considered an infestation and will not pass inspection (Hooper-Bui, 2002). In order to ensure that no IFA are present, the APHIS IFA
Laboratory has established guidelines and authorizes the use of several pesticides for the treatment of regulated articles using several treatment techniques.

Currently, chemicals approved for use in the quarantine program are bifenthrin [2-methylbiphenyl-3-ylmethyl (Z)-(1RS)-cis-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate; FMC Corporation], chlorpyrifos [O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate], diazinon [O,O-diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate], fenoxycarb [ethyl (2-[4-phenoxyphenoxy]ethyl) carbamate; Syngenta Crop Protection, Inc.], fipronil [(+)-5-amino-1-(2,6-dichloro-α,α,α-trifluoro-p-toly)-4-trifluoromethylsulfinyl-pyrazole-3-carbonitrile; Rhone-Poulenc, Bayer Environmental Science, and Aventis], hydramethylnon [tetrahydro-5,5-dimethyl-2(1H)-pyrimidinone [3-4-(trifluoromethyl)phenyl]-1-[2-[4-(trifluoromethyl) phenyl] ethenyl]-2-propenyldiene] hydrazone; BASF Corporation Agricultural Products Group and Waterbury Companies, Inc.], methoprene [isopropyl (2E, 4E, 7S)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate], pyriproxyfen [2-[1-methyl-2-(4-phenoxyphenoxy) ethoxy]pyridine; Valent USA Corporation and Zeneca Group Company], and tefluthrin [2,3,5,6-tetrafluoro-4-methylphenyl) methyl [1,3(z)]=3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate; Uniroyal Chemical Company, Inc.]. These chemicals are sold under the trade names Talstar® (bifenthrin), Dursban® (chlorpyrifos), Diazinon/D-z-n® (diazinon), Award® Bait (fenoxycarb), Chipco® Choice™ (fipronil), Chipco® Top Choice™ (fipronil), Amdro®Pro (hydramethylnon), Siege®Pro Bait (hydramethylnon), Extinguish® Bait (methoprene), Distance® Bait (pyriproxyfen), and Fireban® (tefluthrin).
Application methods for approved chemicals vary based on the regulated items being treated, chemical formulation, duration of certification, and situation in which they are used. The application methods currently approved for the nursery industry are immersion, drenching, topical application, incorporation of granular insecticide into potting media in which containerized plants are grown, in-field treatment for ball and burlap stock prior to harvest, and the fire-ant-free nursery program for containerized plants (Callcott, 2003). Current treatment protocols are available from USDA APHIS.

Bifenthrin and fipronil are the chemicals most recently approved by USDA APHIS, and the Environmental Protection Agency (EPA) for use in the IFA quarantine. Bifenthrin may be applied as a granular or flowable formulation and fipronil may only be applied as a granular formulation (Callcott, 2003).

The agricultural use of fipronil in Louisiana has recently come under scrutiny due to a decline in crawfish (*Procambarus clarkii*) production believed to be associated with Icon™ (fipronil) treated drill-seeded rice in fields that are double-cropped or rotated with crawfish. Recent studies conducted by Louisiana State University have confirmed that Icon™ is detrimental to crawfish (Ottea, 2003). Research indicates that small crawfish are eight times more likely to be affected by high levels of fipronil than larger crawfish and larger crawfish become more susceptible to Icon™ with increases in water temperature.

1.2.5 Historical Control of Imported Fire Ants

Efforts to treat imported fire ant infestations began in 1937 using powdered calcium cyanide [Ca(CN)$_2$] (Williams *et al.*, 2001). Treatment was halted during World War II, allowing infestations to spread. Resumption of treatment in 1948 utilized
chlordane (1,2,4,5,6,7,8,8-octochloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-1h-indene). In 1957, heptachlor (1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene) and dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethanonaphthalene) came into use. However, due to environmental concerns, the use of these chemicals was curtailed. Research then focused on the use of baits. Beginning in the 1960s, baits containing mirex (1,1a,2,2,3,3a,4,5,5a,5b,6-dodecachloro-octahydro-1,3,4-metheno-1h-cyclobuta (cd) pentalene) were widely used and eventually 140 million acres (56 million ha) were treated with mirex baits. The use of mirex baits ended during the 1970s because it was found to be carcinogenic. Hydramethylnon baits were approved for use in 1980 and are still in use today. Between 1958 and 1998, the USDA evaluated over 7200 chemicals for the control of imported fire ants (Williams et al., 2001). Few of these chemicals have shown promise and only a very few have made it to market.

1.2.6 Irrigation Systems

Supplemental irrigation is essential to the production of containerized nursery stock of uniform quality. Fluctuations in natural precipitation are the primary reason for the use of supplemental irrigation. Numerous irrigation system designs exist and are currently in use in the nursery industry. These include ebb-and-flow irrigation, low volume (drip and micro) irrigation and overhead irrigation (Anonymous, 1997).

Ebb and flow irrigation (sub-irrigation) systems, usually used in greenhouse production, utilize water containment barriers (pools), in which nursery stock is grown. Irrigation water held in a separate reservoir is pumped into the water containment barrier until the base of the nursery stock container is submerged. Water is then
absorbed into the container substrate. After irrigation, the water is returned to the reservoir. In comparison to overhead irrigation systems, ebb and flow irrigation systems have a higher initial cost and require a detailed knowledge of water quality, fertility, and disease management; however they require less water and fertilizers because the water and nutrients it contains are recycled (Anonymous, 1997).

Low-volume irrigation (micro-irrigation) systems efficiently emit low volumes of water (in terms of gallons per hour) to a localized area or individual container through drip, spray or micro-sprinkler emitters. In comparison to overhead irrigation systems, low volume irrigation systems have higher initial and maintenance costs; however, savings are realized in the use of smaller pumps and pipe sizes (Anonymous, 1997 and Anonymous, 1994).

Overhead irrigation systems are most commonly used for the production of containerized nursery stock. In these systems, large volumes of water (in terms of gallons per minute) are applied to entire zones in a container yard through impact, spinner or fixed-head nozzles located above the canopy of the crop. In comparison to ebb-and-flow irrigation and low-volume irrigation systems, overhead irrigation systems have a lower initial cost; however, water use is much less efficient due to the amount of water that enters containers verses the amount that falls on the ground between containers. As a result, runoff from overhead irrigation systems can be a major concern (Anonymous, 1997).

In all irrigation systems, efficient irrigation practices are necessary. Factors that influence efficacy are: uniformity of application, substrate water-holding capacity, and the amount of water that enters containers verses the amount that falls between
containers (irrigation efficiency). Proper management of container irrigation is directly related to fertilizer and pesticide run-off (Anonymous, 1997).

The reduction of leachate and run-off can be achieved by increasing irrigation efficiency, which is economically and environmentally important (Yeager et al., 1997). Cyclic irrigation is the division of a volume of irrigation into two or more applications per day (Beeson and Knox, 1991). Research indicates that cyclic irrigation reduces the amount of leachate and run-off from nurseries (Gray, 1999 and Thaxton, 1994).

1.2.7 Potting Media

There are numerous terms used to describe potting media including: soil, media, medium, potting soil, potting mix, container soil, container mix, planting mix, substrate, substratum, dirt, etc. (Anonymous, 1997). In addition the potting media used today are often termed "soilless", "lightweight", or "artificial", because they generally do not contain natural mineral soils (Bunt, 1986). These nursery container substrates serve four functions: to provide water, supply nutrients, permit gas exchange to and from the roots of plants (rhizosphere), and provide mechanical support. Potting media used in nursery production are generally formulations of two or more components. The formulations of potting media vary regionally due to availability, cost, shipping, regional preference, and the growth requirements of different plant species. Common horticultural media components can be divided into two groups: organic and inorganic. Organic media components include: wood products, processed animal and municipal wastes, composts, organic residues, and agricultural by-products. Inorganic components include: sand, silt, clay, perlite, vermiculite, styrofoam, etc. (Bilderback, 1982). These components when combined to create a "soilless" medium have chemical
and physical properties that are very different from natural mineral soils (Bunt, 1986). Potting mixes are generally amended with dolomitic limestone, micronutrients, and fertilizers. Dolomitic limestone amendments are used to supply Ca and Mg and adjust the media pH to a range acceptable for the species being grown. Micronutrients and fertilizer amendments provide the elements essential for plant growth (Anonymous, 1997).

1.2.8 Pine Bark Media

Pine bark, mainly from loblolly and slash pine, is a common nursery potting medium component used in Louisiana and the southeastern U.S. Pine bark is not phytotoxic and may be used fresh as a potting medium, however aging and composting for a period of 9 to 12 months produces a more desirable potting medium. Aging and composting pine back allows larger particles to break down, wood to decompose, and compounds similar to turpentine to degrade. The bulk density of pine bark varies from 0.19 to 0.24 g•cc\(^{-1}\) based on particle size distribution. Pine bark that is not composted or aged has less than 20 to 30% fine particles (=0.5 mm). Fine particles are accountable for potting media moisture retention. Aged pine bark typically has a cation exchange capacity (CEC) of 10.6 meq•100 ml\(^{-1}\). However, the CEC of "soilless" media does not influence growth to the same degree as it does in natural mineral soils. In addition to pine bark, the most widely used potting media components are sand and sphagnum peat moss (Anonymous, 1997).
1.2.9 Potting Media Analysis

As a result of these differences in chemical and physical properties of natural mineral soils and "soilless" media, the analytical methods used to evaluate natural mineral soils are not appropriate to use for the analysis of "soilless" media (Bunt, 1986). The physical make-up of potting media can be divided into three phases: solid, liquid, and gas. The solid phase of potting media can be expressed in terms of the weight of substrate per unit volume of substrate particles (g•cc\(^{-1}\)) or "bulk density". The soil bulk density is affected by substrate particle size distribution. The liquid phase of potting media is expressed in terms of substrate water holding capacity or "container capacity". This is the maximum volume of water held by the substrate after irrigation and drainage. Particle size distribution affects the container water holding capacity: the smaller the particle size distribution the greater the water holding capacity. The gas phase of potting media is expressed in terms of pore spaces. The "total porosity" is a measure of the pore space volume in potting media and is a percentage of total potting medium volume. There is a direct correlation between porosity and water holding capacity: the higher the porosity the higher the water holding capacity (Anonymous, 1997). The chemical properties of "soilless" media are directly related to the chemical makeup of its components.

In research, saturated media extracts and displaced soil solutions (leachates) are most often used for the chemical analysis of potting mixes (Bunt, 1986). The Virginia Tech Extraction Method (pour-through or leachate collection method) is considered a best management practice for the analysis of container substrates (Anonymous, 1997).
1.2.10 Bifenthrin

Bifenthrin (Fig. 1.2, Table A-1, and Table A-2) is a Type II (Anonymous, 2004) fourth-generation pyrethroid (Fig. 1.2) manufactured by FMC Corporation. Pesticides containing bifenthrin as the active ingredient include: Talstar,® Brigade,® Capture,® Torant,® and Zipac.® Pyrethroid insecticides originated in the late 1940s from efforts to synthesize natural pyrethrins derived from chrysanthemum plants. Pyrethroids are lipophilic esters of chrysanthemic acid and are divided into Type I and Type II compounds based on alcohol substituents. Type I compounds contain noncyano alcohol substituents such as descyano-3-phenoxybenzyl or other alcohols. However, the nonphenoxybenzyl alcohol compounds are unstable in the environment and degrade quickly. When Type I compounds contain phenoxybenzyl and halogenated alcohols they are more stable. Type II compounds specifically contain an α-cyano-3-phenoxybenzyl alcohol that greatly increases insecticidal activity. Type II compounds also include an altered phenyl ring in the acid section of the molecule (Ottea, 2002).

Pyrethroids affect the sodium ion channel in both the peripheral and central nervous system of insects, initially stimulating nerve cells and eventually causing paralysis (Ware, 2000).

1.2.11 Environmental Fate of Bifenthrin

The environmental fate of bifenthrin is a direct result of its chemical properties and the biotic and abiotic factors to which it is exposed. The major biotic pathway of bifenthrin degradation is hydrolysis into 4’-hydroxy bifenthrin (Fecko, 1999). Minor pathways of biotic degradation include ester cleavage, hydroxylation, and oxidation into BP acid, BP alcohol, and BP aldehyde. In aqueous environments bifenthrin is usually
adsorbed onto sediment and suspended particles. In addition, hydrolysis forms 4’-hydroxy bifenthrin. In soils, the major degradation pathway involves the formation of 4’-hydroxy bifenthrin and photolysis and ester cleavage produce BP acid, BP alcohol, and TFP acid.

1.2.12 Fipronil

Fipronil is a pesticide in a new family of insecticides called phenyl pyrazoles (Fig. 1.3). It was developed in 1987 at the Rhone-Poulenc Research Station in Ongar, England (Anonymous, 1996a). Fipronil entered the world market in 1993, and was registered for use in the U.S. in 1996. In 1997 production of fipronil was estimated to be 480 tonnes per annum and was expected to rise to 800 tonnes per annum by 2000. Fipronil is labeled for use in a wide range of crops and is effective against a wide range of insect pests. It has been evaluated on over 250 insect pests on more than 60 crops worldwide (Anonymous, 2004d). Pesticides containing fipronil as an active ingredient include: Ascend, Goliath, Nexa, Adonis, Frontline,® Frontline® Top Spot, Regent®
corn insecticide, Icon FS® rice insecticide, Chipco® Choice™ insecticide for mole crickets, Termidor® termiticide, Combat®, MaxForce®, Chipco® Fire Star, Chipco® TopChoice, etc. Fipronil was recently added to the list of authorized insecticides for treatment of nursery stock in accordance with the USDA APHIS Imported Fire Ant Quarantine guidelines (Callcott, 2003).

Granular fipronil is incorporated into potting media based on the dry weight bulk density of potting media and the desired certification period. The following application rates are used in the quarantine: 10 ppm for 6 months certification, 12 ppm for 12 months, 15 ppm for 24 months, and 25 ppm for continuous certification (Callcott, 2003).

![Chemical structure of fipronil](image)

**Fig. 1.3.** Chemical structure of fipronil (Bobé et al., 1997).
Fipronil inhibits the passage of chloride ions by binding with the gamma-aminobutyric acid (GABA)-regulated chloride ion channel within the neurons of the central nervous system of insects. This blockage results in hyper-stimulation of the central nervous system, hyperactivity, convulsions, paralysis and death of the treated insect (Anonymous, 1996b).

1.2.13 Environmental Fate of Fipronil

The fate of fipronil in the environment depends on the formulation, the substrate to which it is applied, and environmental conditions. Fipronil may be applied as a soil treatment, seed treatment, or foliar treatment (Anonymous, 1996b). Rhône-Poulenc reported that laboratory and field studies revealed five main degradation products of fipronil as a result of reduction, oxidation, hydrolysis, and photolysis. The degradation products are MB45950 (sulfide) (Fig. 1.4.) formed through reduction in soil, RPA200766 (amide) formed by hydrolysis in soil or water, MB46513 (Fig. 1.4.) formed by photolysis in water or on soil, and MB46136 (sulfone) (Fig 1.4.) formed through oxidation in soil and which can be further degraded by photolysis in water or on soil into RPA104615 (chemical type not specified). The CAS names for chemical structures in degradation pathways are: desulfinyl photodegradate = 1-H-pyrazole-3-carbonitrile, 5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-trifluoromethyl; sulfone = 1-H-pyrazole-3-carbonitrile, 5-amino-1-[2,6-dichloro-4-[(trifluoromethyl)sulfonyl]; sulfide = 1-H-pyrazole-3-carboxylic acid, 5-amino-1-[2,6-dichloro-4-[(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfonyl]; carboxylic acid = 1-H-pyrazole-3-carboxylic acid, 5-amino-1-[2,6-dichloro-4-[(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfonyl]; amide = 1-H-pyrazole-3-carboxylic acid, 5-amino-1-[2,6-
dichloro-4-[(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfinyl] carboxamide = 1-H-
pyrazole-3-carboxylic acid, 5-amino-1-[2,6-dichloro-4-[(trifluoromethyl)phenyl]-4-
[(trifluoromethyl)] (Connelly, 2001). Further reports of field and laboratory soil
adsorption/desorption and leachate studies, by Rhône-Poulenc (Anonymous, 1996a),
have shown that fipronil and its degradation products have low soil mobility (studies
were conducted using natural soils) and that the risk of contamination to ground water is
low. The solubility of fipronil in water is 1.9 to 2.4 mg•L\(^{-1}\) at 20\(^\circ\)C.

Fig. 1.4. Chemical structures of fipronil metabolites (Ibrahim, 2000).

Research by Bobé et al. (1998) on the hydrolysis and photolysis of fipronil
indicated that the degradation of fipronil on the surface of two soils in aqueous solutions
in the absence of light at ambient temperatures (22 ± 2\(^\circ\)C) is affected by pH. Fipronil is
stable in acid (pH 5.5) and neutral (pH 7.0) solutions with 80% remaining unchanged
after 2400h; however, its degradation sharply increases as pH increases. The half-life ($t_{1/2}$) of fipronil: at pH 9.0 $t_{1/2} = 770h$, at pH 10.0 $t_{1/2} = 114h$, at pH 11.0 $t_{1/2} = 11h$, at pH 12.0 $t_{1/2} = 2.4h$. Furthermore, at pH 5.5, fipronil in aqueous solution exposed to simulated sunlight degraded faster over time ($t_{1/2} = 4.1h$).
CHAPTER 2: MATERIALS AND METHODS

2.1 Introduction

This study was conducted on the Louisiana State University campus at the Department of Horticulture’s Hill Farm Teaching Facility container production yard located in Baton Rouge, La. Potting medium samples and potting medium leachate samples were collected over a period of 180 d. Gas chromatography and mass spectrophotometry were used to measure the levels of bifenthrin, fipronil, and the metabolites of fipronil (MB45950, MB46136, MB46513). The study was initiated on 30 Dec. 2002 and terminated on 28 June 2003.

2.2 Potting Media Preparation

The potting medium used in this experiment consisted of 90% pine bark [1.59 cm screened (5/8 inch)] and 10% mason sand by volume. No amendments (lime, fertilizer, etc.) were added to this media. The components of this medium were uniformly mixed in a 1.53 m³ (2 yd³) commercial soil mixer (Bouldin & Lawson, McMinnville, TN; Model No. 12203).

2.3 Determination of Dry Weight Bulk Density of Potting Medium

A 7 L sample of potting medium was placed in a convection oven at 112°C (250°F) for a period of 4 h. After drying, five 1 L loosely packed samples were measured and weighed (Callcott, 1991). The bulk density of the potting medium was determined to be 0.288 g/(cm³)⁻¹ (Appendix 8).
2.4 Chemical Treatment of Potting Medium

The pesticide treatments used in this study included an untreated control, Talstar® PL Granular Insecticide (0.2% bifenthrin) incorporated into the potting medium at a rate of 10ppm (40.2 g formulated material/0.0283 m$^3$ soil), and Chipco® Top Choice™ Insecticide (0.0143% fipronil) was incorporated into potting medium at a rate of 10 ppm (562 g formulated material/0.0283 m$^3$ soil). These rates were based on the potting medium bulk density (0.288 g•cm$^{-3}$). Potting medium was uniformly mixed in 0.0283 m$^3$ (1 ft$^3$) increments using a 0.06 m$^3$ (2 ft$^3$) concrete mixer (Red Lion, Monarch Industries, Winnipeg, Man.; Model Big Cat type B). Following pesticide incorporation, the media were transferred into standard 3.785 L (1 gal) blow-molded pots (Nursery Supplies Inc., Kissimmee, Fla., Classic® #1, Product ID No. C-400).

2.5 Irrigation System Specifications

Low-volume drip irrigation was used in this study to reduce or eliminate wastewater. The pH of irrigation water was 8.5 with an alkalinity of 200 ml/L. Two irrigation frequencies were used: 1 inch ($\approx$ 586 mL) water pot$^{-1}$ day$^{-1}$ applied once per day and 1/3 inch ($\approx$195mL) water pot$^{-1}$ day$^{-1}$ applied three times per day. The 1 X treatment was applied at 6:00 a.m. and the 3 X treatments were applied at 6:30 a.m., 12:00 a.m., and 5:30 p.m. (equal amounts of water were applied at each application time). Irrigation system components included: Netafim® USA Percision Irrigation™ 1.0 GPH Woodpecker pressure compensating drippers with snout, Netafim® 4-way manifold, Angle Dripper, 125x185V Ft vinyl tubing, PE 0.5 inch tubing, and standard schedule 40 PVC components.
2.6 Sample Collection

Potting medium samples and potting medium leachate samples were collected over a 180 d period on days 1, 30, 60, 120, and 180. The collection dates were 30 Dec. 2002; 29 Jan. 2003; 28 Feb. 2003; 29 Apr. 2003; and 28 June 2003, respectively. Potting medium samples were collected from the top of pots and stored at –30°C until processed. Climatic conditions were recorded during this study (Table A-4 and Table A-5).

Leachate samples were collected using the Virginia Tech Extraction Method (Wright, 1984; 1986). Samples were taken in the morning one hour following the end of the 1 X irrigation treatment and one hour after the first cycle of the 3 X irrigation treatment. Leachate temperatures were not recorded at the time of collection. Pots from which samples were collected, were placed on a closed-capture effluent collection system (modified from Bush et al., 2003) and 300 mL of double distilled deionized water (pH \( \approx 7.0 \), ambient temperature) were poured onto the soil surface to displace the soil solution. The pH of leachate collected from potting media controls, were measured monthly. Leachate was transferred into 250 mL amber glass jars and stored at –86°C (–123°F) until processed.

2.7 Closed–Capture Irrigation Effluent System

The closed capture irrigation effluent system (Fig. 3.1) consisted of a standard 1 gallon pot fitted with a square 35 x 35 cm rubber gasket cut from poly butyl rubber with a circle cut out of the center so that the gasket fit tightly just above the drain holes of the pot. The gasket was large enough to cover the entire system so that all precipitation
was excluded. The gasket was glued to the pot using Multi-Mist™ Fix-All™ multi-purpose adhesive and sealant (Multi-Mist Products, NCH Corporation, Irving, Texas). The pot was placed onto the grate of a 2 L (2 Qt.) oil drain pan (Blitz U.S.A., Inc., Miami, Okla., Model No. 11837) fitted with a 30 x 50 cm (12 x 20 inch) polyethylene bag. The grate was placed deep enough in the bag to allow the top of the bag to reach the bottom of the gasket just above the drain holes of the pot. The bag and grate were placed into the oil drain pan.

2.8 Pesticide Extraction From Potting Medium Leachate Samples

The procedure used to extract bifenthrin and fipronil from leachate samples was adapted from a procedure used to extract most pesticides from water except for the acid herbicide group (Ward, 2004). A leachate sample was removed from storage and allowed to reach ambient temperature. The sample was poured into a 100 mL graduated cylinder, the volume measured and recorded, and the sample transferred into a 500 mL separatory funnel fitted with a PTFE stopcock. Thirty-five mL of methylene chloride were measured in the same graduated cylinder and gently swirled to remove sample residue. The methylene chloride was then transferred to the storage bottle in which the leachate sample had been. The bottle was capped and shaken vigorously for 30 s to remove sample residue. The methylene chloride was then transferred to the 500 mL separatory funnel containing the sample. The separatory funnel was capped and shaken vigorously for 1.5 min and gas was vented by inverting the funnel and opening the stopcock every 15 to 20 s. The funnel was racked and the contents allowed time to separate. During the separation period, a 100 mm glass 60° bowl funnel was prepared.
Fig. 2.1. Diagram of the closed-capture irrigation effluent system used in this experiment (modified from Bush et al., 2003). 1. pot, 2. location where gasket is fitted to pot, 3. drain holes, 4. rubber gasket, 5. oil drain pan
by plugging the stem with a small amount of glass wool and rinsing it with 3 mL of hexane. The bowl of the funnel was filled one quarter full with sodium sulfate (ACS certified, 10 to 60 mesh, prepared by rinsing with petroleum ether and allowed to dry under a hood). Following separation the methylene chloride layer in the separatory funnel was drained through the prepared funnel containing the sodium sulfate into a beaker. Then 35 mL of methylene chloride were measured and added to the remaining sample contained in the separatory funnel and it was extracted a second time as described above. The beaker containing the methylene chloride mixtures from both extractions was placed in a water bath set between 40 and 50°C and evaporated until almost dry. Three mL of hexane was then added and also evaporated until the residue was almost dry. The hexane was added to replace the methylene chloride for analysis. During the evaporation period, a 35 mm glass 58° bowl funnel was prepared by inserting a small amount of glass wool into the bowl, rinsing with hexane, and filling the bowl half full with sodium sulfate prepared as described previously. The sample was transferred to a 15mL graduated centrifuge tube by rinsing the residue with 2 to 3 mL of hexane 3 to 4 times pouring the rinsate through the 35 mm prepared funnel each time. The volume of the centrifuge tube was raised to approximately 12 mL. The centrifuge tube was sealed with a cork (VWR No.23420-184, size 6, grade XXXX) that was rinsed three times with petroleum ether. The sample was stored at room temperature until analyzed. Each time a set of samples was extracted a blank sample and a spiked sample were processed in the same manner as described above. The blank sample consisted of 250 mL distilled water and 40 mL of methylene chloride. The
spiked sample consisted of 250 mL of distilled water, 40 mL of methylene chloride, and 1mL of analytical grade pesticide standard (either bifenthrin or a mixture of fipronil, MB45950, MB46136 and MB46513). The spiked samples were used to calculate percent recovery (Fields et al., 1997).

2.9 Pesticide Extraction From Nursery Media Samples

A Dionex Accelerated Solvent Extractor (Model No. 200) was used to extract bifenthrin and fipronil from the nursery medium. Samples were removed from storage and allowed to reach ambient temperature. Each sample was placed on a 30 x 30 cm sheet of aluminum foil under a hood and allowed to dry at ambient temperature. Drying time was approximately 48 h. The dried samples were ground using an ultra-centrifugal mill (Retsch ZM1, 0.5 mm sieve). Ground samples were stored in paper food service cans at –30°C. Extraction cells (33 mL, stainless steel, Dionex #048763) were fitted with cellulose filters (Dionex #049458) and each was filled with a ground sample. If excess space was present in the extraction cell, it was filled with Ottawa sand. Samples were extracted with ethyl acetate (nanograde). After each run the extract was placed under nitrogen (Zymark Turbo Vap® LV) and evaporated to a volume of 2 to 3 mL. During the evaporation period, a 35 mm glass 58° bowl funnel was prepared by inserting a small amount of glass wool into the bowl, rinsing with hexane, and filling the bowl half full with sodium sulfate prepared as previously described. The evaporated extract was transferred into a 15 mL graduated centrifuge tube by rinsing the collection vial with 2 to 3 mL of ethyl acetate 3 to 4 times, pouring the rinsate through the prepared funnel each time. The volume of the centrifuge tube was raised to
approximately 12 mL. The centrifuge tube was sealed with a cork (prepared by rinsing three times with petroleum ether) and stored at ambient temperature until analyzed (Berggren et al., 1998).

2.10 Operating Conditions of Apparatuses Used for Sample Analyses

Analyses of all extracted potting medium leachate samples were conducted using gas chromatography with dual electron capture detection (GC/dual ECD) and results were verified using gas chromatography mass spectrometry in single ion monitoring mode (GCMS). Potting medium samples were analyzed using only GCMS in single ion monitoring mode.

The GC/dual ECD apparatuses used were a Hewlett Packard 6890 GC, a Hewlett Packard 7673 Autoinjector. Rtx®-CLPesticides (30 m, 0.53 mm ID, 0.5 μm) and Rtx®-CLPesticides2 (30 m, 0.53 mm ID, 0.42 μm) capillary columns (Restek Corporation, Bellefonte, Pa.) were used for simultaneous dual-column confirmation. The system software used was HP GC Chem Station rev. A.06.01 (403). The GC operating conditions were: oven (program) initial temperature was 80°C and held for 1 min, ramped 30°C/min to 190°C, ramped 3.6°C/min to 260°C, held for 2 min; inlet temperature was 270°C; Helium was used as a carrier gas with a flow rate of 1.0 mL/min. The ECD temperature was 350°C with nitrogen used as a make up gas. Retention times varied (Table 2.1) for each compound.

The GCMS apparatuses used were a Hewlett Packard 6890 GC equipped with a Hewlett Packard 5972 MSD (Agilent Technologies, Palo Alto, Calif.), a Hewlett Packard 7673 Autoinjector, and DB-35MS (30 m, 0.25 mm, 0.15 μm) capillary
column ((J&W Scientific (Agilent Technologies)). The software used by this system was HP

Table 2.1. Approximate sample retention times for analyses of select compounds via GC dual ECD.

<table>
<thead>
<tr>
<th>Compound</th>
<th>ECD 1 column: Restek Rtx®-CL Pesticides1. Retention time (min.)</th>
<th>ECD 2 column: Restek Rtx®-CL Pesticides 2. Retention time (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fipronil</td>
<td>14.3</td>
<td>13.6</td>
</tr>
<tr>
<td>MB45950</td>
<td>13.8</td>
<td>13.4</td>
</tr>
<tr>
<td>MB46136</td>
<td>18.2</td>
<td>16.8</td>
</tr>
<tr>
<td>MB46513</td>
<td>11.7</td>
<td>10.9</td>
</tr>
<tr>
<td>Bifenthrin</td>
<td>16.003</td>
<td>19.021</td>
</tr>
</tbody>
</table>

GC Chem Station G1701BA ver. B.01.00. The GC operating conditions were:
oven (program) initial temperature 80°C for 1 min, ramped 20°C/min to 190°C, ramped
8°C/min to 260°C, hold 5 min; inlet temperature was 265°C; Helium was used as a
carrier gas with flow rate of 1.0 mL/min. The MS operating conditions were: transfer
line temperature was 280°C. The MSD was tuned with maximum sensitivity autotune
using PFTB. The EM volatage was set by the tune (≈2500). The solvent delay was 8
min. The MS acquisition parameters used follow: acquisition mode, electron ionization
(EI) in selected ion monitoring mode (SIM). The retention time and masses of the
target ion, qualifier 1 ion and qualifier 2 ion varied for each compound that was
analyzed for (Table 2.2).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention Time (min)</th>
<th>Target</th>
<th>Qualifier 1</th>
<th>Qualifier 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fipronil</td>
<td>9.87</td>
<td>366.9</td>
<td>368.9</td>
<td>212.8</td>
</tr>
<tr>
<td>MB45950</td>
<td>9.73</td>
<td>350.9</td>
<td>352.9</td>
<td>254.9</td>
</tr>
<tr>
<td>MB46136</td>
<td>11.31</td>
<td>382.8</td>
<td>384.8</td>
<td>240.8</td>
</tr>
<tr>
<td>MB46513</td>
<td>8.46</td>
<td>387.9</td>
<td>332.9</td>
<td>280.9</td>
</tr>
<tr>
<td>Bifenthrin</td>
<td>8.21</td>
<td>180.95</td>
<td>165.0</td>
<td>166.0</td>
</tr>
</tbody>
</table>

2.11 Analyses

The procedures used for the analyses of samples for GC/ECD and GCMS were the same. Prior to analyses, bifenthrin samples were dried to a common volume under nitrogen. For fipronil samples, two dilutions were used to account for differences in concentrations of fipronil and fipronil metabolites. The injection volume was 2 µL. The system was primed by injecting fipronil standards (≈16 mg•L⁻¹) three times for GC/ECD and GCMS. Prior to running samples, the column was loaded with injections of seven different concentrations of standard mixes (Appendix 6). The standard injections were used to make the calibration curve (minimum accepted coefficient of determination was ≥0.98) from which values of sample peaks were calculated. If sample results did not fit the calibration curve, the dilutions of samples were changed to
fit the curve or a higher calibration curve was used. If correlation fell below this level, first integration parameters were checked, then the inlet liner and the gold seal were replaced, and the sample reanalyzed. Potting media extracts were suspended in ethyl acetate and leachate samples in hexanes. Potting media sample extracts were dried to a minimum of 5 mL and suspected positives were dried to 10 mL. Results of analyses were adjusted mathematically to account for differences in sample weights, volumes and any necessary dilutions. Blanks (hexane) were run between each sample to prevent carry over. Every 4 to 5 samples, one point from the calibration curve was run as an unknown as a system check. Every ten to twelve samples the entire standard set was rerun and a new calibration curve calculated. Spiked samples were used to calculate percent recovery (Fields, 2001).

Chromatograms and reports of analytical results are on file at the La. State Univ. AgCenter, Dept. of Agr. Chem., 102 Agricultural Chemistry Building, Baton Rouge, LA 70803. Records are maintained for ten years.

2.12 Procedure for Cleaning Glassware Contaminated with Fipronil

Due to the possibility of fipronil adhering to glassware, all glassware used for the extraction and analysis of fipronil was decontaminated by first washing it in hot soapy water, and then triple rinsing it (hot tap water followed by deionized water followed by methanol or acetone). After rinsing, the glassware was placed on a rack and allowed to air dry. The glassware was then baked in an oven using the following protocol: raise from room temperature to 450°C within one hour, hold at 450°C for a period of three hours, then cool to 25°C within two hours (Ibrahim et al., 2000).
2.13 Experimental Design

Treatments were arranged in a randomized complete block design (Appendix 7), with 6 blocks for each of the harvest dates totaling 252 pots. Separate pots were used for each harvest date.

2.14 Statistical Analysis of Data

Data were analyzed using the SAS® System for Windows ver. 9.0 (SAS Institute, Raleigh, NC) using the Proc GLM and Proc Means subprograms. Graphs were created using SigmaPlot® 2001 for Windows ver. 7.0 (SPSS, Inc., Chicago, IL).
CHAPTER 3: RESULTS, DISCUSSION, AND CONCLUSIONS

3.1 Irrigation

There were no significant differences ($p = 0.05$) in bifenthrin, fipronil, MB 45950, MB 46136, and MB 46513 levels detected between irrigation treatments (1 X and 3 X) in either potting medium or potting medium leachate, therefore data were pooled for statistical analyses.

It was believed that there would be significant differences in the levels of bifenthrin, fipronil, MB 45950, MB 46136, and MB 46513 detected between irrigation treatments in both potting medium and potting medium leachate. Cyclical irrigation is known to reduce the amount of leachate and runoff from container grown plants (Gray, 1999). It was probable that containers which received cyclical irrigation would have higher levels of these chemicals in soil and leachate samples, in comparison to containers that receive the same amount of water at one time interval, because less would have left the pot in leachate and runoff.

3.2 Bifenthrin Levels in Potting Medium and Potting Medium Leachate

In this analysis, no bifenthrin was detected in the untreated potting medium controls. Concentrations of bifenthrin in potting medium samples collected from potting medium treated with bifenthrin were 8.41, 4.94, 5.42, 4.36, and 4.88 ppm at 1, 30, 60, 120, and 180 d, respectively. The level of bifenthrin in the potting medium at 1 d was greater than all other sampling days. The level of bifenthrin in the soil remained constant from 30 to 180 d (Fig. 3.1).
It is probable that the level of bifenthrin was greatest at 1d because it had not bound to soil particles at this point in time and was easily extracted from the soil.

During the study, no bifenthrin was detected in the leachate samples collected from the untreated potting medium controls. The concentrations of bifenthrin in leachate samples collected from potting medium treated with bifenthrin were 57.33, 19.83, 8.2, 1.82, and 4.14 ppb at 1, 30, 60, 120, and 180 d, respectively. The level of bifenthrin in leachate samples at 1 d was greater than all other sampling days. Bifenthrin levels at 30 d were greater than levels from 60 to 180 d. Levels of bifenthrin at 120 d were less than levels at 60 and 180 d (Fig. 3.2).

Fig. 3.1. Mean concentrations of bifenthrin in potting medium samples over the 180 d period. (Error bars represent SE, n=12).
Fig. 3.2 Mean concentrations of bifenthrin in potting medium leachate samples over 180 d period. (Error bars represent SE, n=12. No error bar indicates that SE was smaller than diameter of symbol).
3.3 Fipronil Levels in Potting Medium and Potting Medium Leachate

For the period of the study no fipronil was detected in the untreated potting medium controls. Concentrations of fipronil in samples collected from potting medium treated with fipronil were 8.03, 9.23, 4.77, 3.18, and 3.12 ppm at 1, 30, 60, 120, and 180 d, respectively. The concentrations of fipronil at 1 and 30 d were greater than all other sampling days. The fipronil level at 60 d was greater than the levels at 120 and 180 d. The level of fipronil remained constant between 120 and 180 d (Fig. 3.3).

![Graph showing fipronil concentrations over time](image)

**Fig. 3.3.** Mean concentrations of fipronil in potting medium samples over 180 d period. (Bars represent SE, n=12).
For the duration of the study, no fipronil was detected in leachate samples collected from untreated potting medium controls. The concentrations of fipronil in leachate samples collected from potting medium treated with fipronil were 40.08, 36.75, 28.00, 14.92, and 19.50 ppb at 1, 30, 60, 120, and 180 d, respectively. The concentrations of fipronil at 1 and 30 d were larger than all other collection dates. The fipronil concentration at 60 d was greater than 120 and 180 d. Fipronil concentrations remained constant between 120 and 180 d (Fig. 3.4).

**Fig. 3.4.** Mean concentrations of fipronil in potting medium leachate samples over 180 d period. (Error bars represent SE, n=12).
3.4 MB45950 Levels Potting Medium and Potting Medium Leachate

Throughout the study, no MB45950 was detected in untreated potting medium controls. The concentrations of MB45950 in potting medium treated with fipronil were 0.54, 0.22, 0.03, 0.21, and 0.27 ppm at 1, 30, 60, 120, and 180 d, respectively. The concentration of MB45950 in potting medium at 1 d was greater than all other collection days. The level of MB45950 was lowest at 60 d. Levels detected at 30, 120, and 180 d were not significantly different from each other and were higher than 60 d (Fig. 3.5).

Fig. 3.5. Mean concentrations of MB45950 in potting medium samples over 180 d period. (Error bars represent SE, n=12).
During this study, no MB45950 was detected in leachate samples collected from untreated potting medium controls. The concentrations of MB45950 in leachate collected from potting medium treated with fipronil were 0, 0.27, 0.7, 1.2, and 9.75 ppb at 1, 30, 60, 120, and 180 d, respectively. The concentrations of MB45950 at 1, 30, and 60 d were not different from one another. The mean concentration at 120 d was not different from 60 d but was different from all other collection dates. The concentration of MB45950 at 180 d was greater than all other collection dates (Fig. 3.6).

Fig. 3.6. Mean concentrations of MB45950 in potting medium leachate samples over 180 d period. (Error bars represent SE, n=12. No bar indicates that SE was smaller than diameter of symbol).
3.5 MB46136 Levels in Potting Medium and Potting Medium Leachate

Throughout this study, no MB46136 was detected in untreated potting medium controls. The concentrations of MB46136 in samples collected from potting medium treated with fipronil were 0.76, 0.93, 0.92, 0.91, and 1.03 ppm at 1, 30, 60, 120, and 180 d, respectively. The concentration of MB46136 at 1 d was significantly less than the mean concentration at 180 d. There were no differences in concentrations of MB46136 between 1, 30, 60, and 120 d. There were no differences between levels or MB46136 in potting medium samples collected at 30, 60, 120, and 180 d (Fig. 3.7).

Fig. 3.7. Concentrations of MB46136 in potting medium samples over 180 d period. (Error bars represent SE, n=12).
No MB46136 was detected in leachate collected from untreated potting medium controls during this study. The concentrations of MB46136 in leachate collected from potting medium samples were 0.99, 1.4, 2.37, 2.62, and 3.2 ppm at 1, 30, 60, 120, and 180 d, respectively. The lowest concentrations were detected at 1 and 30 d. The concentration at 60 and 120d were greater than the concentrations at 1 and 30 d. The concentration of MB46136 detected at 180 d was greater than all other collection dates (Fig. 3.8).

Fig. 3.8. Concentrations of MB46136 in potting medium leachate samples over 180 d period. (Bars represent SE, n=12).
3.6 MB46513 Levels in Potting Medium and Potting Medium Leachate

No MB46513 was detected in potting medium or potting medium leachate during the 180 d study.

3.7 Discussion

This study was undertaken to examine the fate of bifenthrin and fipronil incorporated into nursery media composed of 90% pine bark and 10% sand for the control of imported fire ants. In addition, two irrigation regimes (1 X and 3 X) were evaluated to determine their affect on the fate of bifenthrin and fipronil in this media. Nursery media samples and nursery media leachate samples were tested to evaluate changes in the levels of bifenthrin, fipronil, and fipronil metabolites (MB 45950, MB 46136, and MB 46513) over a six-month period.

This study indicates that the irrigation regimes used in this research did not influence the fate of these chemicals. Bifenthrin levels in nursery potting media initially dropped and then remained constant throughout this study. As expected, fipronil levels in nursery potting media decreased over time during this study. The bifenthrin levels found in leachate collected from nursery potting media were higher than the known solubility of bifenthrin in water (less than 0.1 ppb) and the levels of fipronil found in leachate collected from nursery potting media were higher than the solubility of fipronil in water (1.9 to 2.4 ppb). It is likely that the increased levels of bifenthrin and fipronil were due to the high levels of organic acids, phenols, terpenes, resins and natural solvents found in pine bark, which may increase the solubility of these pesticides. In addition, small organic particles suspended in the leachate samples may have increased the levels of bifenthrin and fipronil detected. During this study,
there were no indications that temperatures influenced the fate of bifenthrin and fipronil. The metabolite of fipronil, MB46513, was not detected during this study in potting media or potting media leachate. MB46513 is a product of photolysis in soil and water (Anonymous, 1996a). Fipronil was incorporated into the potting media in this experiment, which limited its exposure to sunlight; it is probable this is why MB46513 was not detected. MB46136 levels in potting media initially increased and then remained constant over time, while levels of this metabolite in media leachate increased over time. Levels of the MB45950 in potting media fluctuated over time, while levels of this metabolite increased over time in media leachate. MB45950 is a product of reduction in soil and MB46136 is a product of oxidation in soil. Changes in the levels of these metabolites over time may be attributed to increases in the degradation of fipronil in potting media over time.

The potting media temperatures and the temperatures of the leachates (at the time of collection) were not monitored during this experiment and it is possible that fluctuations in these temperatures influenced the fate of bifenthrin and fipronil. In future research the aforementioned factors should be addressed. However, atmospheric conditions (Tables A-4 and A-5) did not indicate a noticeable trend.

Previous research has shown the pH of aqueous solutions influences the half-life of fipronil (Bobé et al., 1998). It is possible that the high pH and alkalinity (8.5 and 200 ml/L) of the irrigation water used in this experiment increased the degradation of fipronil. Previous research indicates that the alkalinity of irrigation water affects the pH of both leachates and potting media. The carbonates and bicarbonates found in alkaline water may raise the pH of potting media and leachates over time (Gray, 1999). The pH
of potting media was not measured during this research. In this research, there were no indications that the pH of irrigation water affected the pH of leachates over time. Measurements taken during this research (samples temperature 21°C) showed that the pH of leachates ranged from 5.5 to 7.2 and the average pH of leachates was 6.5. There were no significant differences in pH between sampling dates.

Future research which may further explain the fate of bifenthrin and fipronil in nursery potting media should focus on identifying the chemical components found in pine bark leachate and determining how they affect the solubility of these two chemicals in water.

3.8 Conclusions

In this research, irrigation frequency did not affect the levels of bifenthrin, fipronil, and fipronil metabolites found in potting media and media leachates, so data were pooled for statistical analyses. Bifenthrin levels in the potting media initially dropped and then remained constant throughout the study. Fipronil levels in nursery potting media decreased over time. Levels of the fipronil metabolite MB45950 in potting media fluctuated over time, while the levels of this metabolite increased over time in media leachates. Levels of the fipronil metabolite MB46136 in potting medium increased slightly and then remained constant over time, while levels of this metabolite increased over time in medium leachates. The metabolite of fipronil, MB46513, was not detected during this study in potting medium or medium leachates. Levels of bifenthrin and fipronil detected in potting media leachates were higher than expected. It is likely that these increased levels were due to the high levels of organic compounds found in pine bark media leachates. It is also possible that the pH and temperature of
media, leachates, and irrigation water affected the fate of these chemicals in this research, however it was not apparent in this study. This research confirmed that bifenthrin and fipronil are bound tightly by organic soils at levels sufficient to control of imported fire ants.


### APPENDIX 1
CHEMICAL AND PHYSICAL PROPERTIES OF BIFENTHRIN AND FIPRONIL

Table A-1. Chemical and physical properties of bifenthrin and fipronil.

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Bifenthrin(^z)</th>
<th>Fipronil(^y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer</td>
<td>FMC Corporation</td>
<td>Rhône-Poulenc, Bayer, Aventis</td>
</tr>
<tr>
<td>CAS number</td>
<td>82657-04-3</td>
<td></td>
</tr>
<tr>
<td>Chemical Name</td>
<td>2-methylbiphenyl-3-ylmethyl (Z)-(1RS)-cis-3-(2-chloro-3,3,3-trifluoroprop-1-yl)-2,2-dimethylcyclopropanecarboxylate</td>
<td>(+)-5-amino-1-(2,6-dichloro-(\alpha,\alpha,\alpha)-trifluoro-p-toly)-4-trifluoromethylsulfinyl-pyrazole-3-carbonitrile</td>
</tr>
<tr>
<td>Chemical Family</td>
<td>pyrethroid</td>
<td>phenyl pyrazole</td>
</tr>
<tr>
<td>Empirical Formula</td>
<td>(C_{23}H_{22}ClF_{3}O_{2})</td>
<td>(C_{12}H_{4}Cl_{2}F_{6}N_{4}OS)</td>
</tr>
<tr>
<td>Molecular Weight</td>
<td>422.88</td>
<td>437.1</td>
</tr>
<tr>
<td>Henry's constant</td>
<td>7.20 (\times 10^{-3}) atm m(^3)•mol(^{-1}) at 25°C</td>
<td>3.7 (\times 10^{-4})</td>
</tr>
<tr>
<td>Appearance</td>
<td>off-white to pale tan waxy solid, viscous liquid</td>
<td>white powder at 23°C</td>
</tr>
<tr>
<td>Odor</td>
<td>very faint, slightly sweet</td>
<td>moldy smell at 23°C</td>
</tr>
<tr>
<td>Melting Point</td>
<td>68 to 70.6°C</td>
<td>195.5-203°C (technical grade), 200 to 201°C</td>
</tr>
<tr>
<td>Specific Gravity</td>
<td>1.212 g•mL(^{-1}) at 25°C</td>
<td>1.480 to 1.629 at 20°C</td>
</tr>
<tr>
<td>Density</td>
<td>1.39 g•mL(^{-1})</td>
<td>1.44 to 1.626 g•mL(^{-1}) at 20°C</td>
</tr>
<tr>
<td>Octanol/Water Partition Coefficient</td>
<td>(K_{ow}=1x10^{6}) (log Kow &gt;6)</td>
<td>log Po/w=4.00</td>
</tr>
<tr>
<td>Vapor Pressure</td>
<td>0.024 mPa (1.81 (\times 10^{-7}) mm Hg at 25°C)</td>
<td>3.7 (\times 10^{-9}) hPa (2.8 (\times 10^{-9}) mm Hg)</td>
</tr>
</tbody>
</table>

\(^z\)Fecko, 1999.

\(^y\)Anonymous, 1996b.
APPENDIX 2
SOLUBILITY DATA

Table A-2. The solubility of bifenthrin and fipronil in selected solvents.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Solubility of bifenthrin$^z$</th>
<th>Solubility of fipronil$^y$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0.1 mg/L less than 0.1 ppb</td>
<td>1.9-2.4 mg/l at 20°C</td>
</tr>
<tr>
<td>Acetone</td>
<td>Yes</td>
<td>545.9 g/l</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>no data</td>
<td>22.3 g/l</td>
</tr>
<tr>
<td>Ethyl Acetate</td>
<td>no data</td>
<td>264.9 g/l</td>
</tr>
<tr>
<td>Hexane</td>
<td>no data</td>
<td>28 mg/l</td>
</tr>
<tr>
<td>Methanol</td>
<td>Slightly</td>
<td>137.5 g/l</td>
</tr>
<tr>
<td>1-Octanol</td>
<td>no data</td>
<td>12.2 g/l</td>
</tr>
<tr>
<td>2-Propanol</td>
<td>no data</td>
<td>36.2 g/l</td>
</tr>
<tr>
<td>Toluene</td>
<td>Yes</td>
<td>3.0 g/l</td>
</tr>
</tbody>
</table>

$^z$ Anonymous, 1996b.
Table A-3. Toxicity of bifenthrin and fipronil to select species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Bifenthrin $^z$</th>
<th>Fipronil $^y$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat (LD$_{50}$)</td>
<td>54/70 mg•kg$^{-1}$ (male/female)</td>
<td>97 mg•kg$^{-1}$</td>
</tr>
<tr>
<td>Bonwhite quail (LD$_{50}$)</td>
<td>1800 mg•kg$^{-1}$</td>
<td>11.3 mg•kg$^{-1}$</td>
</tr>
<tr>
<td>Mallard (LD$_{50}$)</td>
<td>2150 mg•kg$^{-1}$</td>
<td>&gt;2150 mg•kg$^{-1}$</td>
</tr>
<tr>
<td>Bluegill sunfish (LC$_{50}$)</td>
<td>0.00035 ppm</td>
<td>0.085 ppm</td>
</tr>
<tr>
<td>Daphnia carinata (LC$_{50}$)</td>
<td>0.00160 ppm</td>
<td>3.8 ppm</td>
</tr>
<tr>
<td>Honey bee (contact)</td>
<td>not available</td>
<td>0.00593 µg•bee$^{-1}$</td>
</tr>
</tbody>
</table>

$^y$ Anonymous, 1996b.
APPENDIX 4
WEATHER DATA

Table A-4. Climatological data by month.

<table>
<thead>
<tr>
<th>Month</th>
<th>Average temperature (°C)</th>
<th>Total precipitation (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>December 2002</td>
<td>11.1</td>
<td>18.16</td>
</tr>
<tr>
<td>January 2003</td>
<td>8.4</td>
<td>1.32</td>
</tr>
<tr>
<td>February 2003</td>
<td>11.6</td>
<td>18.44</td>
</tr>
<tr>
<td>March 2003</td>
<td>16.1</td>
<td>5.44</td>
</tr>
<tr>
<td>April 2003</td>
<td>19.6</td>
<td>8.08</td>
</tr>
<tr>
<td>May 2003</td>
<td>25.5</td>
<td>1.14</td>
</tr>
<tr>
<td>June 2003</td>
<td>26.8</td>
<td>18.75</td>
</tr>
</tbody>
</table>

Table A-5. Climatological data on sample collection dates.

<table>
<thead>
<tr>
<th>Sample collection date</th>
<th>Average temperature (°C)</th>
<th>Min temperature (°C)</th>
<th>Max temperature (°C)</th>
<th>Total precipitation (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-30-2002</td>
<td>17</td>
<td>11</td>
<td>22</td>
<td>trace</td>
</tr>
<tr>
<td>01-29-2003</td>
<td>20</td>
<td>16</td>
<td>24</td>
<td>0.69</td>
</tr>
<tr>
<td>02-28-2003</td>
<td>9</td>
<td>7</td>
<td>11</td>
<td>trace</td>
</tr>
<tr>
<td>04-29-2003</td>
<td>22</td>
<td>14</td>
<td>29</td>
<td>0</td>
</tr>
<tr>
<td>06-28-2003</td>
<td>27</td>
<td>22</td>
<td>31</td>
<td>trace</td>
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</table>
APPENDIX 5
IRRIGATION AND PRECIPITATION DATA

Table A-6. Total monthly irrigation, precipitation and combined total (irrigation + precipitation) received by experimental units.

<table>
<thead>
<tr>
<th>Month</th>
<th>Total irrigation (cm)</th>
<th>Total precipitation (cm)</th>
<th>Combined total (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>December 2002</td>
<td>5.1</td>
<td>2.97</td>
<td>8.07</td>
</tr>
<tr>
<td>January 2003</td>
<td>78.7</td>
<td>1.32</td>
<td>80.02</td>
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<tr>
<td>February 2003</td>
<td>71.1</td>
<td>18.44</td>
<td>89.56</td>
</tr>
<tr>
<td>March 2003</td>
<td>78.7</td>
<td>5.44</td>
<td>84.18</td>
</tr>
<tr>
<td>April 2003</td>
<td>76.2</td>
<td>8.08</td>
<td>84.28</td>
</tr>
<tr>
<td>May 2003</td>
<td>78.7</td>
<td>1.14</td>
<td>79.88</td>
</tr>
<tr>
<td>June 2003</td>
<td>71.1</td>
<td>18.75</td>
<td>85.19</td>
</tr>
</tbody>
</table>
### APPENDIX 6
### ANALYTICAL STANDARD DATA

Table A-7. Concentrations of analytical standard mixes (ng/µg) used before October 2003.

<table>
<thead>
<tr>
<th>Compound</th>
<th>0.034</th>
<th>0.067</th>
<th>0.167</th>
<th>0.335</th>
<th>0.669</th>
<th>1.510</th>
<th>3.010</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fipronil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MB45950</td>
<td>0.035</td>
<td>0.070</td>
<td>0.175</td>
<td>0.349</td>
<td>0.698</td>
<td>1.570</td>
<td>3.140</td>
</tr>
<tr>
<td>MB46136</td>
<td>0.032</td>
<td>0.064</td>
<td>0.159</td>
<td>0.319</td>
<td>0.638</td>
<td>1.430</td>
<td>2.870</td>
</tr>
<tr>
<td>MB46513</td>
<td>0.041</td>
<td>0.081</td>
<td>0.203</td>
<td>0.406</td>
<td>0.812</td>
<td>1.830</td>
<td>3.650</td>
</tr>
</tbody>
</table>

Table A-8. Concentrations of analytical standard mixes (ng/µg) used after October 2003.

<table>
<thead>
<tr>
<th>Compound</th>
<th>0.033</th>
<th>0.065</th>
<th>0.130</th>
<th>0.326</th>
<th>0.653</th>
<th>1.310</th>
<th>3.270</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fipronil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MB45950</td>
<td>0.033</td>
<td>0.065</td>
<td>0.130</td>
<td>0.327</td>
<td>0.654</td>
<td>1.310</td>
<td>3.270</td>
</tr>
<tr>
<td>MB46136</td>
<td>0.030</td>
<td>0.061</td>
<td>0.121</td>
<td>0.301</td>
<td>0.603</td>
<td>1.210</td>
<td>3.030</td>
</tr>
<tr>
<td>MB46513</td>
<td>0.031</td>
<td>0.061</td>
<td>0.122</td>
<td>0.307</td>
<td>0.614</td>
<td>1.230</td>
<td>3.070</td>
</tr>
</tbody>
</table>
Table A-9. Diagram illustrating the placement of pots and treatments used to assess the retention of bifenthrin and fipronil by nursery potting medium. The layout represents a randomized complete block design with six replicates.

<table>
<thead>
<tr>
<th>C₁W₁R₁</th>
<th>C₁W₁R₂</th>
<th>C₁W₁R₃</th>
<th>C₁W₁R₄</th>
<th>C₁W₁R₅</th>
<th>C₁W₁R₆</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₆</td>
<td>T₄</td>
<td>T₂</td>
<td>T₄</td>
<td>T₂</td>
<td>T₁</td>
</tr>
<tr>
<td>T₁</td>
<td>T₀</td>
<td>T₄</td>
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<td>T₂</td>
<td>T₀</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>C₁W₂R₀</th>
<th>C₁W₂R₁</th>
<th>C₁W₂R₂</th>
<th>C₁W₂R₃</th>
<th>C₁W₂R₄</th>
<th>C₁W₂R₅</th>
</tr>
</thead>
<tbody>
<tr>
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<td>T₂</td>
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<td>T₂</td>
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<td>T₁</td>
<td>T₂</td>
<td>T₁</td>
<td>T₂</td>
<td>T₀</td>
</tr>
<tr>
<td>T₄</td>
<td>T₂</td>
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Treatments: C₁ = fipronil, C₂ = bifenthrin, C₃ = untreated control, W₁ = water 1t/day, W₂ = water 3t/day. R = replicate. Harvest dates: T₀=1d, T₁=30d, T₂=60d, T₃=120d, T₄=180d.

## APPENDIX 8
### POTTING MEDIA BULK DENSITY DATA

Table A-10. Potting medium weights used in this experiment for the determination of potting medium dry weight bulk density.

<table>
<thead>
<tr>
<th>Beaker Number</th>
<th>Beaker Weight (g)</th>
<th>Weight of Beaker + Sample (g)</th>
<th>Sample Weight (g)</th>
<th>Sample Bulk Density (g•cm$^3$)</th>
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<tbody>
<tr>
<td>1</td>
<td>380.85</td>
<td>648.35</td>
<td>267.50</td>
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<td>2</td>
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<td>3</td>
<td>384.35</td>
<td>679.35</td>
<td>295.00</td>
<td>0.2950</td>
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<td>4</td>
<td>381.05</td>
<td>661.80</td>
<td>280.75</td>
<td>0.2808</td>
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<td>5</td>
<td>379.88</td>
<td>674.38</td>
<td>294.50</td>
<td>0.2945</td>
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</table>

Average bulk density of 5 samples = 0.288g•cm$^3$
VITA

Russell Stanley Harris, III, son of Russell S. Harris, Jr. and Earlita L. Harris, was born in New Orleans, Louisiana, on 23 July 1973. He is a graduate of Robert Mills Lusher Elementary School and Eleanor Laura McMain Magnet Secondary School in New Orleans, Louisiana. He entered college in the fall of 1991. During his undergraduate studies Mr. Harris attended the University of New Orleans in Louisiana, the Harvard Ukrainian Research Institute in Cambridge, Massachusetts, and the National Agricultural University of Ukraine in Kiev. Mr. Harris completed his undergraduate studies at Louisiana State University Agricultural and Mechanical College in Baton Rouge and was graduated from there in December of 1997 with a Bachelor of Science degree in horticultural systems. Following graduation he worked as a horticulturist and manager in the golf course industry for three and one half years prior to his enrollment in the Graduate School at LSU in the fall of 2001. Mr. Harris is a candidate for the degree of Master of Science in horticulture at LSU.