THE INFLUENCE OF SOILLESS GROWING MEDIA ON THE MOVEMENT AND LONGEVITY OF THE HERBICIDE DIMETHENAMID-P

by

ALEXANDRA PERSEVERANDA WILLIAMS

(Under the direction of Mark Czarnota)

ABSTRACT

Pine bark based soilless growing media dominates substrates use in containerized plant production in the ornamental industry. Due to high organic matter content coupled with limited cation exchange capacity (CEC), surface area, and bulk density, the time period herbicides can provide weed control within soilless media combinations may vary. Limited information exists in scientific literature about the interaction of soilless growing media with herbicides. Dimethenamid-P was recently registered by BASF for control of annual grasses, broadleaf weeds, and sedges in containerized ornamentals. A study was conducted to examine dimethenamid-P chemical behavior for two soilless media, pine bark alone, and a commercial mix containing pine bark. Data indicated that dimethenamid-P was not detected in the leachate from these materials. Bioassay data indicated that dimethenamid-P grown in the pine bark mix provided significant control of common lambsquarters (*Chenopodium album*) and tall fescue (*Festuca arundinacea*) for 6 weeks after treatment.

INDEX WORDS**:** *Chenopodium album*, chloroacetamide, Dimethenamid-P, dose response, *Festuca arundinacea*, herbicide leaching, pine bark, repeated bioassay, soilless growing media

THE INFLUENCE OF SOILLESS GROWING MEDIA ON THE MOVEMENT AND LONGEVITY OF THE HERBICIDE DIMETHENAMID-P

by

ALEXANDRA PERSEVERANDA WILLIAMS

B.S., Western Kentucky University, 2006

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements of the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2009

THE INFLUENCE OF SOILLESS GROWING MEDIA ON THE MOVEMENT AND LONGEVITY OF THE HERBICIDE DIMETHENAMID-P

by

ALEXANDRA PERSEVERANDA WILLIAMS

Major Professor: Mark Czarnota

Committee: Scott NeSmith

Timothy Grey

Clint Waltz

Electronic Version Approved:

Maureen Grasso

Dean of the Graduate School

The University of Georgia

December 2009

DEDICATION

This work is dedicated to my loving and supporting family. Thank you for believing in me, encouraging me, and providing me with an abundance of love throughout my life. Life is not easy, but with a loving family, God, and a whole lot of patience, all things are possible. I love you all very much!

ACKNOWLEDGEMENTS

I would like to first and foremost thank Dr. Czarnota for his continuous support throughout my studies at UGA. The guidance, generosity, and amount of care that you have provided for me; the lessons I have learned; the words of advice; the things to avoid; the stories you have told; and the risks to take; I've absorbed them all and they will forever be implanted in my mind and I will carry these experiences and words with me for the rest of my life. I also want to extend an additional thank you to my committee members. Dr. NeSmith, thank you for stopping by to check in with me. Even if it was just to say hello, it meant so much to have your support. Dr. Grey, thank you so much for your time and efforts. Even though we are far away, I have always felt that I could call you up if I needed to because we share a common thing, we are Kentuckians! Dr. Waltz, thank you for your honest opinions and your attention to detail. Your guidance has really opened my eyes to the reality of the science world, and for that I am grateful. Thank you to all of the students in the Horticulture Department and the friends from Griffin Experiment Station that I have made. You guys are the ones that have kept me sane! And finally I want to thank LaMont Sudduth for his hard work that he has put into helping me complete my research. Thanks for your goofy antics. They have made my time in Griffin, GA so memorable. I will never forget you all!

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

Chapter 1. Introduction

The production of container grown ornamentals has increased substantially within the last several decades, revolutionizing the nursery business (Anonymous, 2004). In 2006, container nurseries comprised 27.6% of the total ornamental nurseries in the state of Georgia (Boatright and McKissick, 2006). Georgia ornamental horticulture production generated $765 million in sales in 2006 (Boatright and McKissick, 2006). In a 2007 survey, the United States value of sales for nursery stock was approximately $6.5 billion (USDA, 2007). Growing plants in containers creates a convenient marketing package that allows for easy transport, saves space, and enables the grower to select a suitable growing medium (primarily light-weight soilless substrates); many of which are composed of bark residuals (Simmons and Derr, 2007; Ingram et al., 1991). Pine bark is the main component utilized for the production of nursery crops in the southeast, partly due to its availability (Fain et al., 2003; Wehtje et al., 2009).

Despite the advantages of growing ornamentals in containers, weeds are still a problem. Weeds compete for water, nutrients, light, container space, and may increase insects, diseases, and vertebrate pest populations (Altland, 2003; Wallace and Hodges, 2007). Weeds infest containers via wind and irrigation (Horowitz and Elmore, 1991), and are introduced by human traffic from contaminated clothing and shoes (Stamps, 1997). Currently, the best defense at preventing and selectively controlling weed growth in containerized nurseries is the utilization of preemergence (PRE) herbicides (Czarnota, 2008). Hand weeding large scale nurseries is impractical, inefficient, and expensive (Billeaud and Zajicek, 1989; Gallitano and Skroch, 1993). Herbicides, when applied according to recommended label rates provide PRE weed control, and

help nursery growers produce high quality marketable container crops. Marketable nursery crop production requires an effective integrated weed management program, including: weed seed removal from pots and around growing areas, using weed free media and propagation stock, and utilization of PRE herbicides. When PRE herbicides are applied at recommended label rates, they can be valuable weed control tools that help maintain the value of a nursery crop (Altland, et al., 2007).

Current recommendations for most herbicide applications for containerized ornamentals are typically every 60 to 90 days. However, many Georgia growers report that weeds continue to emerge beyond 60 days (Czarnota, 2008). Registration labels limit the quantity of herbicide that can be applied in a given time period. However, this practice is ineffective and of no impact if adequate weed control cannot be attained.

Over the past five years, several herbicides used alone, or in combinations, have become available to the containerized nursery industry. One of the most recent herbicides registered for ornamental weed control is dimethenamid-P-, [-(*S*)-2-chloro-*N*-[(1-methyl-2-methoxy) ethyl]-*N*-(2,4-dimethyl-thien-3-yl)- acetamide].

Leaching is a concern following application of container ornamental herbicides. Leaching of PRE herbicides can result in premature loss of weed control, damage to the containerized ornamental, and has the potential to cause environmental contamination (Grey et al., 1996). Several parameters determine the leaching potential of a herbicide. The physical and chemical characteristics of a growing media and herbicide, the amount of irrigation water applied, and media temperature all contribute to the leaching potential (Peter and Weber, 1985; Landis et al., 1991). For many reasons, including leaching potential and groundwater contamination, the Environmental Protection Agency (EPA), United States Department of

Agriculture (USDA), and /or the National Agricultural Pesticide Impact Assessment Program (NAPIAP) will usually restrict the amount (kg/ha) of active ingredient that can be applied in a 12 month period. Moreover, some states currently ban the use of certain active ingredients because of ground water contamination concerns (i.e 2,4-D and other phenoxy herbicides) (Szmedra, 1997). Presently, dimethenamid-P is limited to 3.36 kg ai/ha per growing season.

Purpose of Study

The objectve of this study was to determine the leaching potential and longevity of dimethenamid-P when applied to containers of two commercial pine bark media mixes. Information from this study could provide a better understanding of the amount of dimethenamid-P lost to leaching in the growing media. In addition, this study could provide needed information on the length of control to be expected with dimethenamid-P.

Chapter 2. Literature Review

History of Ornamentals

Anywhere humans have traveled, plants and flora have followed (Nelson, 1985). The intentional human use of plants for aesthetic and functional purposes dates back to the hanging gardens of Babylon (Acquaah, 2005). In 1530, the first book written about nurseries, *Seminarium,* was created by author, Charles Estienne (Hartmann, 2002). In the 1600's, The Netherlands were the chief supplier and international headquarters for the nursery trade (Nelson, 1985). Their reputation and capabilities soon spread to the royal courts in Europe, and shortly thereafter, The Netherlands became providers of luxuries such as spring flowers in the winter and out of season fruit (Nelson, 1985).

The first nursery in the United States has been credited to William Prince and Son and was located in Long Island in the early 1730s (Hartmann, 2002). In the 19th century, the nursery business expanded throughout the eastern region of the United States (Hartmann, 2002). Later, nurseries catered to not only the wealthy, but the everyday gardener as well. Modern nurseries facilitate the work of landscape architects, contractors, and home gardeners by selling plant materials that are ready to be installed in a landscape (Acquaah, 2005). Support from academic programs in institutions of higher learning along with research in public and private sectors have allowed for further advancements in ornamental horticulture (Acquaah, 2005). Together, these advances have led to other technologies and practices that have helped shape modern day plant nurseries. Today, the art of landscaping with ornamentals is considered a fundamental element of modern home construction (Acquaah, 2005). Now anyone can enjoy the spectacular fragrance

of *Clethera alnifolia* or the shade and beauty of a majestic *Ginko biloba* thanks to centuries of work by dedicated nurserymen and women.

Weed Problems in Containers and Herbicide Control

In its broadest definition, a weed is a plant that is undesirable in a given space. Weeds are a nuisance because they can outgrow field crops, take over natural habitats, and potentially cause harm to people or animals (i.e. poison ivy, *Toxicodendron radicans*). Weeds can become established in containers just as they do in agronomic settings due to unsanitary media, contaminated irrigation water, diffusion by wind, or seed dispersal mechanisms. Hand weeding in a large scale nursery is impractical, inefficient, and expensive (Billeaud and Zajicek, 1989; Gallitano and Skroch, 1993). Padgett and Frazier (1962) conducted at study in Georgia and determined that 1542 hours of manual labor were required to weed 1 ha of marketable container stock. With a current $7.25 minimum wage, it would be estimated to cost $11, 180/ha for manual weeding, which is economically impractical.

To remain profitable, it is imperative that nurseries find effective integrated weed management programs. Programs should include sanitation, use of weed free propagation sources, and the judicious use of herbicides. Even with the use of herbicides, weed species such as, *Cardamine hirsuta* , *Euphorbia maculata*, *Fatoua villosa*, *Oxalis stricta*, and *Phyllanthus tenellus* continue to be difficult weeds to control. Herbicides in the ornamental nursery have both benefits and limitations. Although they are cost efficient and help decrease crop loss, due to increasing prices of fuel and machinery, the overall cost of making aherbicide application can be will increase too. Therefore, it is important to take all expenses into consideration and to use application timing wisely.

Common Leaching Problems in Containers

Herbicides utilized by the container ornamental industry are often broadcast applied in the form of sprays or granulars in/or around containers (Riley et al., 1994). Herbicide leaching from containers can be caused by excess irrigation, resulting in a "flush" through the substrate and exiting through the pot onto the ground. Leaching can contribute to pollution of surface and ground water, and can eventually reach recycled irrigation water which can lead to levels that are phytotoxic to nursery stock (Horowitz and Elmore, 1991; Keese et al., 1994).

Detectable concentrations of simazine ([6-chloro-*N*,*N*'-diethyl-1,3,5-triazine-2,4-diamine]) and metolachlor ([2-chloro-6'-ethyl-*N*-(2-methoxy-1-methylethyl) acet-*o*toluidine]) have been reported in nursery runoff water (Mahnken et al., 1994). A study by Horowitz and Elmore (1991), evaluated the effect of leaching of oxyfluorfen in four different container media. Their results indicated that leaching depth of oxyfluorfen increased in order from the following media: peat and sand (1:1) mix, Stockton clay soil, Yolo fine sandy loam soil, and redwood bark and sand (3:1) mix; concluding that depth of leaching was not related to media organic matter content. An Alabama study by Keese et al. (1994), applied pendimethalin and oxyfluorfen, oryzalin and oxyfluorfen, and oxadiazon in a container ornamental nursery and reported that residues from these granular herbicides moved from the site of application into runoff water and contaminated on-site ponds. Riley et al. (1994) also conducted a pendimethalin and oxyfluorfen runoff study at a commercial nursery business for two years and found that minimal herbicide residues were found in pond water and sediment during the duration of the study. It was concluded by Riley et al. (1994) that a best management practice to reduce herbicide residues would be to apply reduced herbicide volumes many times during the season, rather than large doses once or twice throughout the year.

Dimethenamid-P Classification, Chemical and Physical Properties

Herbicides are classified by chemical families based on their molecular structure, which determines the herbicide's mode/mechanism of action, or how the herbicide effects or alters a working process in a plant. Dimethenamid-P is grouped with the chloroacetamide herbicides. The chloroacetamide herbicide family can also be referred to as the acetamide, acetanilide, amide, chloroacetanilide, and chloroacetamide families (Retzinger and Mallory-Smith, 1997; Senseman, 2007) in other literary references.

Chloroacetamides were first discovered and developed in the 1950's, and since then agriculture has benefited from their herbicidal control (Hamm 1974). In crop production, chloroacetamides are characterized as being some of the most commonly used herbicide families developed for weed control (Le Baron et al., 1988; Couderchet et al., 1998; Schmalfub et al., 1998).

Dimethenamid-P has a molecular weight of 275.79g/mole, a density of 1.19 g/ml (25C) and is water soluble at 1174 mg/L (25 C) (Senseman, 2007). $C_{12}H_{18}ClNO_2S$ is the molecular formula for dimethenamid-P. Dimethenamid-P has no pK_a since it is non ionic, and has a K_{ow} of 141+/- 6 at 25 C (Senseman, 2007). Dimethenamid-P is dark in appearance and has a faint odor. When combined with other ingredients to make the formulated product (i.e. petroleum distillates and xylene), the odor is strong and tar-like. At 55 C, dimethenamid-P was stable for 90 days in soil (Senseman, 2007). The half life of dimethenamid-P has reportedly ranged from 1-2 weeks in the southern United States to 5-6 weeks in the northern part of the U.S. (Senseman, 2007).

Dimethenamid-P Uses

Dimethenamid-P was traditionally formulated to control specific broadleaf weeds and selective annual grasses in field corn (*Zea mays*). Its uses have been broadened for other

agricultural commodities such as sugarbeet (*Beta vulgaris* L.), cucumber (*Cucumis sativus*), squash (*Cucurbita maxima*), soybean (*Glycine max*), sunflower (*Helianthus annuus*), sweet potato (*Ipomoea batatas*), snapbean (*Phaseolus vulgaris*), potato (*Solanum tuberosum*), and sorghum (*Sorghum spp*.) (Osborne et al., 1995; Riechers et al., 1996; Yokley et al., 2002; Zimmerman, et al., 2002; Anderson et al., 2005; Hutchinson et al., 2005; Bollman and Sprague, 2007). Riechers et al. (1996) found that dimethenamid-P can be used on wheat (*Triticum aestivum*) when combined with a safener and Robinson et al. (2008) reported that sulfentrazone tank-mixed with dimethenamid-P provided suitable weed control in bell pepper (*Capsicum annuum*). Most recently, dimethenamid-P has been formulated for ornamental nursery crops to control common annual grasses and broadleaf weeds and sedges. Due to their mechanism of action, chloroacetamides are predominantly used PRE as their phytotoxic affects can control many grass and broadleaf weeds (Bollman and Sprague, 2007; Bollman and Sprague 2008).

Dimethenamid-P can be applied POST, early preplant (EPP), or preplant incorporated (PPI), all dependent on the crop registration label (Bollman and Sprague, 2008). Dimethenamid-P can be applied as a spray in water or liquid fertilizer, on dry bulk fertilizer, or formulated as a granular (Senseman, 2007).

Dimethenamid-P Behavior in Plants

According to previous research, chloroacetamide herbicides are absorbed primarily through shoots in grasses (Bollman et al., 2008), by the roots in broadleaf plants, (Le Baron et al., 1988), or through cotyledons as they emerge through treated soil (Böger et al., 2000; McGregor et al., 2005). When chloroacetamide herbicides are taken up by the roots, shoots, or cotyledons, they are transported within the susceptible targeted plant via the xylem by acropetal movement, inhibiting early development of the weed (Böger et al., 2000). When weed seeds

germinate in dimethenamid-P treated soil, dimethenmid-P reduces cell division (cytokinesis) which blocks seedling growth and the targeted plant remains stunted and distorted, usually unable to emerge above the soil line (Fuerst, 1987; Böger et al., 2000; McGregor et al., 2005).

Herbicides have the ability to inhibit plant growth by interfering with crucial metabolic or bioenergetic pathways, mostly via specific interactions with an essential target enzyme (Boger, 2003). Dimethenamid-P is thought to have a mechanism-of-action which is an inhibition of very long chain fatty acid (VLCFA) synthesis resulting from 1) an inhibition of all four steps in the elongation step series, 2) increasing inhibition together with the decrease of acyl-CoA primer substrate concentration, and 3) a tight bind between the inhibitor and the targeted enzyme (Schmalfub et al., 2000; Senseman, 2007). Coenzyme A exists in all living cells and is essential for lipid metabolism, which is inhibited by chloroacetamides in plants (Gronwald, 1991). Figure 2.1 illustrates the proposed site of action that dimethenamid-P is thought to inhibit VLCFA synthesis.

VLCFAs are comprised of more than 20 carbon atoms and can be saturated or monounsaturated. They are located in epicuticular waxes, seed storage lipids, and membrane lipids of higher plants (Ebert and Ramsteiner, 1984; Millar and Kunst, 1997; Matthes and Böger, 2002; Leonard et al., 2004). Fatty acids of 20 carbon atoms or less are formed in the plastid (Gotz and Boger, 2004). Many VLCFAs are found within the plasma membrane, particularly sphingolipids (Matthes and Böger, 2002; Gotz and Boger, 2004), and are thought to be synthesized by a microsomal elongase system of the endoplasmic reticulum as well as in the Golgi apparatus (Schmalfub et al., 2000; Boger, 2003). This elongation within the endoplasmic reticulum and/or Golgi apparatus begins with successive elongation of a C18 fatty acyl predecessor by two carbons which originated from malonyl-CoA (Matthes and Böger, 2002).

The four steps (and four enzymes) by which VLCFAs are synthesized by fatty acid elongases are: 1) condensation of malonyl CoA with a long chain acyl CoA (VLCFA-synthase); 2) reduction to β-Ketoacyl CoA (β -Ketoacyl CoA-reductase); 3) β-Hydroxyacyl-CoA dehydration (β-Hydroxyacyl-CoA dehydrogenase); and 4) 2-trans-Enoyl-CoA reduction (2-trans-Enoyl-CoA reductase), ensuing a lengthened acyl CoA (von Wettstein-Knowles, 1982; Fehling and Mukherjee, 1991; Millar and Kunst, 1997; Leonard et al., 2004). Chloroacetamides have demonstrated the inhibition of microsomal fatty acid elongation which results in a decrease in VLCFAs in plant cells (Matthes and Böger, 2002) and a decline in VLCFAs can lead to a collapse of the plant system (Boger, 2003). When the VLCFA synthesis is inhibited, the plasma membrane is disturbed, losing stability and becoming leaky, resulting in a cascade of secondary effects (i.e. decreased cell division) and the eventual death of the susceptible plant (Matthes and Böger, 2002; Eckermann et al., 2003).

Dimethenamid-P as a Stereoisomer

All naturally derived compounds are chiral (Williams, 1996), including DNA, RNA, and proteins (Muller and Kohler, 2004). Some synthetically derived chemicals, including herbicides, can be chiral. Acetamide herbicide compounds can possess different elements of chirality. Chirality refers to a molecule that is not superimposable on its own mirror image (Muller and Kohler, 2004), or that it is impossible for every major feature of two objects to reflect symmetrically. Chiral molecules contribute to nearly 30% of all pesticide usage (EPA, 2007); yet, approximately 7% of the total market value is sold only as single isomers (Williams, 1996). Molecules that are characterized to have chirality are comprised of individual members, which are called enantiomers. An enantiomer of the same compound is one of two or more stereoisomers that appear to have identical physical and chemical properties when examined

under standard analysis (Liu et al., 2005; Wong, 2006). Most chiral compound enantiomers are perceived differently by biochemical processes (Maier et al., 2001; Muller and Kohler, 2004). As concluded by Muller and Kohler (2004), the fate of chiral compound enantiomers in the environment can vary in characteristics like degradation and undesirable side-effects (i.e. enantiomer accumulation in the environment, species, or organ).

Several organic agrochemicals are comprised of stereoisomers (Saito et al., 2008). Dimethenamid, [2-chloro-*N*-[(1-methyl-2-methoxy)ethyl]-*N*-(2,4-dimethyl-thien-3-yl)-acetamide], stereoisomerism results from two types of chiral elements (meaning with two diastereomeric pairs of enantiomers): one being axial (an outcome from the delayed rotation about the phenyl-nitrogen bond and an appropriate asymmetric substitution of the phenyl ring, or 2,4-dimethylthien-3-y1 moiety), and the other being C-chiral (caused by having an asymmetrically substituted C-atom in the alkyl moiety) (Buser and Mueller, 1995; Couderchet et al., 1997; Muller and Kohler, 2004). These two elements of chirality lead to two diastereomeric pairs of enantiomers that are comprised of four stereoisomers : a*S*,l'*S*-, a*R*,l'*S*-,a*R*,l'*R*-, and a*S*,l'*R*-configuration (Buser and Mueller, 1995; Couderchet et al., 1997). Of the four stereoisomers, dimethenamid has two key isomers, or an enantiomeric pair, that can be distinguished as a*RS*,1´*S* and a*RS*,1´*R* (abbreviated *S* and *R* respectively). In efficacy trials conducted by Couderchet et al. (1997), dimethenamid-P, the *S*-isomer of the chemical dimethenamid, was revealed to be the active isomer. Figure 2.2 illustrates the chemical structures of the dimethenamid *S* and *R* isomers. According to the Herbicide Handbook (Senseman, 2007), the *S* isomer refers to *sinister,* meaning left, representing a priority of atoms from highest to lowest, rotating counterclockwise while attached to a chiral center; while *R* is the

symbol meaning *rectus* or right with a clockwise priority of atoms also arranged from highest to lowest, rotating around a chiral center (Senseman, 2007).

Due to high expense, chiral herbicides are traditionally not separated and used as a racemic mixture, or a combination of its enantiomers (Liu et al., 2005). However, if two isomers of a racemic mixture have contradictory degrees of selectivity and interact differentially with other chiral molecules, the manufacturer typically produces the isomer possessing the most advantageous qualities (Williams, 1996; Wong, 2006). Isolating isomers, like dimethenamid-P, means lowering use rates which decreases the chance of chemicals to be released unintentionally into environmental systems (Muller et al., 2001). This is especially valuable for dimethenamid (racemic form) use since it has been shown to move through the environment by runoff and leaching (Muller and Buser, 1995; Kalkhoff et al., 1998; Mueller et al., 1999; Crawford et al., 2002).

Dimethenamid-P Interactions with Soil

Herbicides can react differently in various growing mediums. Degradation rates of herbicides in soil and soilless mediums depend on media texture, media microbial activity, herbicide bioavailability, environmental temperature, available moisture, and organic carbon (Beigel et al., 1999; Ma et al., 2006). Dimethenamid-P adsorption to a growing medium is largely dependent on organic matter and clay content (Bollman and Sprague, 2007). Increased rates of soil organic matter and clay content can decrease rates of herbicide degradation and increase adsorption values in acetanilide herbicides (Zimdahl and Clark, 1982). According to Winton and Weber (1996), pesticide mobility is inclined to be higher in course textured soils with low organic matter and shows the least amount of mobility in soils with fine texture and moderate to high organic matter contents. Bollman et al. (2008) showed that when

dimethenamid-P is applied to soil, the tendency was to bind to organic matter due to a lower K_{oc} (sorption coefficient) value compared to another acetamide herbicide (e.g. *s*-metolochlor). Most acetamide herbicides possess high water solubilities for preemergence herbicides and leach post application, making them one of the most detected classes of herbicides in natural water (Thurman et al., 1992; Wong, 2006).

According to Senseman (2007), dimethenamid-P is moderately adsorbed to the soil, primarily degraded by microbes, and has low losses due to volatilization and photodegradation. Because chloroacetamide herbicides are affected by microbial degradation, their half-lives in the soil are generally short (Zimdahl and Clark, 1982). Dimethenamid-P persistence within the soil proved to have a DT_{50} (half-life) averaging 20 days over 10 field studies conducted in Europe and North America, while in the southern U.S. it ranged from 1-2 weeks (Senseman, 2007). An activity, adsorption, and mobility study of acetanilide and amide herbicides, by Vasilakoglou et al. (2001), determined that lower amounts of dimethenamid-P were adsorbed than the other herbicides evaluated; in consequence, a greater amount of dimethenamid-P was leached through the soil. Mueller et al. (1999) observed that metolachlor has a greater half life than dimethenamid-P. Furthermore in another study by Bollman and Sprague (2007) agreed that metolachlor has a greater residual activity than dimethenamid-P.

Once a herbicide is applied, degradation processes in the soil generate a complex configuration of metabolites (Fava et al., 2000). The major degradation product of dimethenamid-P is oxalamide (Senseman, 2007). A leaching study conducted by Fava et al. (2000) found the two chloroacetamide herbicides, alachlor and metolachlor, have short half lives, are low leaching, and their metabolites in groundwater do not pose a threat to human health . A lab study which investigated the fate of metolachlor metabolites, ESA (ethane sulfonic acid) and

OXA (oxanilic acid), concluded that metolachlor degradation is not enantioselective, and therefore racemization did not occur in either soil or activated sludge (Klein et al., 2006). Studies by Crawford et al. (2002), indicated ^{14}C metabolites accumulated as much as 20% of the applied [^{14}C] dimethenamid in anaerobic redox conditions in the soil environment. Two major metabolites were found in nonautoclaved treatments, while autoclaved microcosms observed just one (Crawford et al., 2002). This study also revealed that greater than 50% of the applied [^{14}C] dimethenamid was eventually integrated into soil-bound residue (Crawford et al., 2002).

Properties of Pine Bark and Other Container Substrates

The function of a container growing medium is to physically support the plant and to continuously provide balanced and sufficient amounts of oxygen, water and nutrients for rooting (Handreck and Black, 2002; Ingram et al., 1991). Most outdoor container nurseries use bark as the main component in combination with one or more materials to form multiple growing medias (Gabriel et al., 2009). Pine bark is the main component utilized for the production of nursery crops in the Southeast because of the strong Southeastern timber market (Fain et al., 2003; Wehtje et al., 2009). For other regions in the United States, such as Oregon, the most common components for soilless media include douglas fir bark, sphagnum peat moss, and pumice, mainly due to the availability of these materials in their area (Gabriel et al., 2009).

Other components used in various horticultural container crops can include perlite, vermiculite, sand, rockwool, organic coir, sphagnum peat, coconut chips, compost, cedar bark, redwood shavings, melaleuca bark (*Melaleuca quinquenervia*), douglas fir bark (*Pseudotsuga menziesii*), scoria (macrovesicular volcanic rock), poppy straw, pumice (igneous rock), peanut hulls, composted sewage sludge, gasifier residue (from burning organic materials), bagasse (a by-product of the sugarcane industry), polystyrene foam, polyphenolic foam, and hydrophillic

gels (Beardsell et al., 1979; Nelson, 1985; Bunt, 1988; Heiskanen, 1999; Atiyeh et al., 2000; Abad, 2001; Blythe and Merhaut, 2007; Buamscha et al., 2007; Gabriel et al., 2009; Ingram et al., 1991). Pine bark maybe used as the single component for container media with no other additives (Simmons and Derr, 2007). The goal for creating an ideal container substrate combination is that it will reduce the cost and management necessary for production (Ingram et al., 1991). Wehtje et al. (2009) found that herbicide rates safe for container ornamentals grown in pine bark were also acceptable in other container substrates including clean chip tree and tree parts.

When compared to field soil, pine bark has a higher infiltration rate because of its large volume of macropores (Simmons and Derr, 2007). Many in the ornamental container industry consider bark a desirable growing media (Laiche and Nash, 1986). Pine bark used for containerized ornamentals is produced by processing it through a hammermill machine in which the bark is pulverized to a desirable consistency and particle size (Laiche and Nash, 1986).

Lysimeter Background and Technique

Lysimeters have been used as significant tools to assess the movement and longevity of pesticides in soils (Sakaliene et al., 2009). The fate of a herbicide in a growing media is dependent on the herbicide's chemical properties as well as the physical and chemical composition of the growing media, which can influence water movement and therefore the path of an herbicide (Sakaliene et al., 2009). Some lysimeter designs can assist in monitoring the effects of hydrolysis, photolysis, soil metabolism , mobility, soil adsorption, and volatility of a chemical compound in a given environment (Winton and Weber, 1996). Two common types of lysimeters used involve an isolated undisturbed segment of a soil profile (a natural monolith) or a disturbed profile (filled-in) which mimics the soil or soilless media intended for analysis; both of

which can have either free drainage or suction-controlled drainage located at the base of the unit (Winton and Weber, 1996; Sakaliene et al., 2009). Materials to create a lysimeter require them to be non-sorptive to pesticides and harmless to soil organisms and vegetation that may be used in a study (Winton and Weber, 1996). Lysimeters have been assembled from stainless steel due to the potential of the herbicide reacting with the lysimeter (i.e. binding with the materials) (Koskinen et al., 1999). Stainless steel is durable, however, it is expensive, heavy, and requires special skills to assemble. Polyvinyl chloride (PVC) is an economical alternative to constructing lysimeters (Koskinen et al., 1999).

Analytical Methods for Detecting and Identifying the Dimethenamid-P Compound

Determining of features like the presence and quantity of herbicides was first conducted using colorimetric and spectrophtometric methods which often did not produce the results of modern technologies (Tadeo et al., 2000). Today, more advanced tools cannot only identify the presence and quantity of herbicide compounds, but can distinguish between the parent compounds and their metabolites (Tadeo et al., 2000). The measurement of optical activity, or a chiral molecules potential to rotate the plane of polarized light, is a key technology in several areas of chemical research, including agricultural biotechnology (Bobbitt and Linder, 2001). Like most stereoisomer chemicals, detecting and quantifying dimethenamid-P by trial and error was often implemented using various methods and technologies. It is important for these methods to be developed so the scientific community can obtain necessary information about the fate of a particular pesticide.

Anderson et al. (2005) developed a method for extracting dimethenamid-P in raw agricultural commodities, including beet (*Beta vulgaris*), cabbage (*Brassica oleracea*), squash (*Cucurbita maxima* and *pepo*), and sweet corn (*Zea mays*) by obtaining samples with a

methanol-water solution, followed by filtration, and then extraction with hexane, which proved to be faster than a methanol-toluene solvent mixture. Separation of samples and quantification of dimethenamid-P was acquired via gas chromatography (GC) with an electron capture detector (Anderson et al., 2005). This method proved to be a quick and efficient way of determining dimethenamid-P concentration levels within agricultural commodities.

Buser and Mueller (1995) conducted a study involving the chromatographic separation of enantiomers and diastereomers of acetamide pesticides, including dimethenamid-P, with the objective of discovering the environmental behavior of these chemicals. Studies indicated that dimethenamid-P analysis using methods such as chiral high performance liquid chromatography (HPLC) or capillary zone electrophoresis using ambient temperatures were better over chiral high resolution gas chromatography (HRGC) due to unstable dimethenamid atropisomers (Buser and Mueller, 1995).

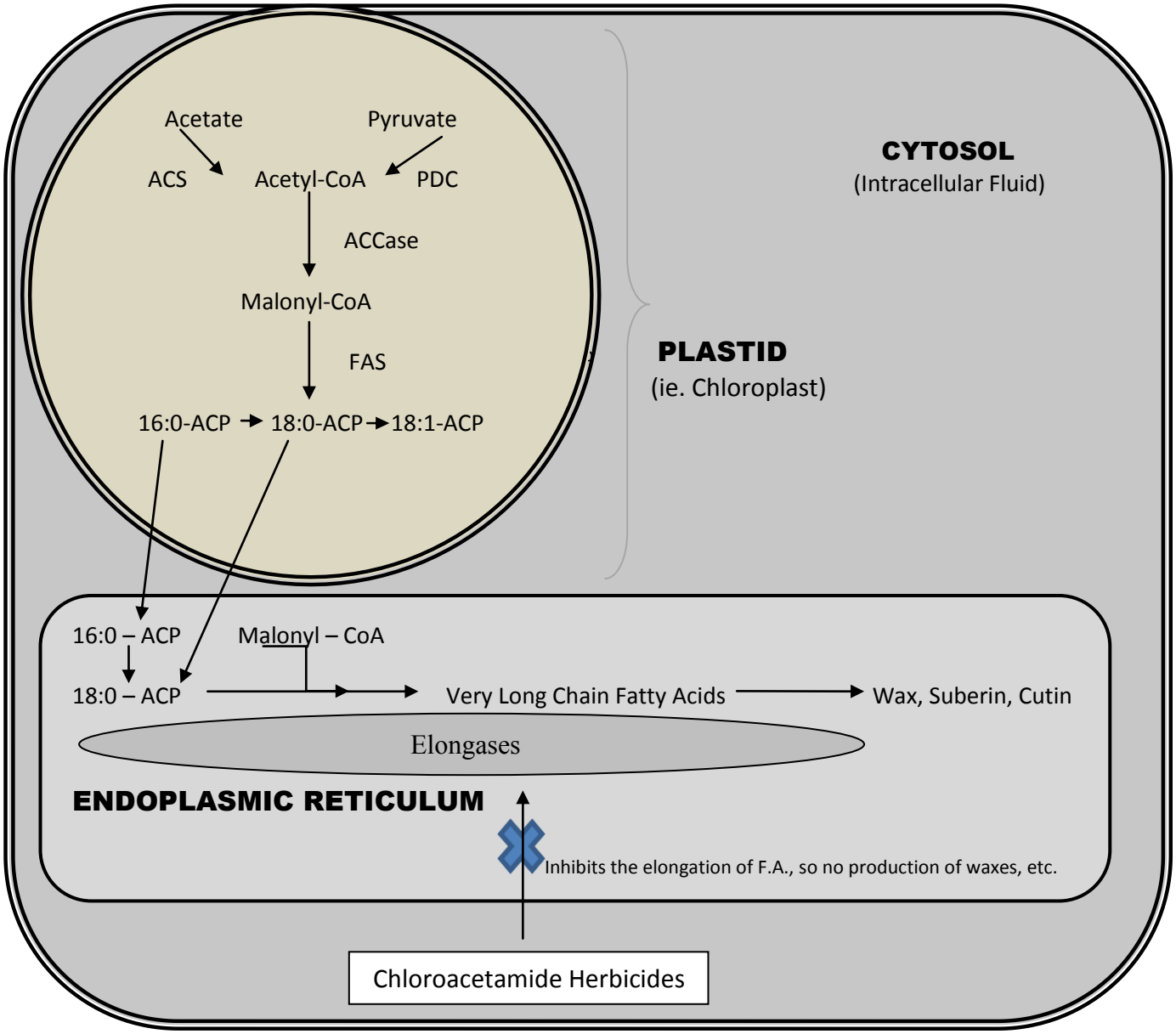


Figure 2.1. A schematic of fatty acid biosynthesis within higher plant cells, demonstrating the proposed site of action for chloroacetamide herbicides. Abbreviations: ACS, acetyl-CoA synthase; PDC, pyruvate dehydrogenase complex; ACCase, acetyl-CoA carboxylase; FAS, fatty acid synthase; ACP, acyl carrier protein. Modified from Gronwald, J.W. 1991. Lipid biosynthesis inhibitors. Weed Sci. 39:435-449.

Figure 2.2. Chemical structures of dimethenamid *S* and *R* forms. (a.) The *(S)* indicates the location of the axial chiral elements. The bold wedge represents the bonds of atoms above the plane of the drawing (as if it were coming out of the paper towards the reader) with the narrow end of the wedge coming from the center of the bond. (b.) The hashed wedged bond represents the bond of atoms below the plane of drawing (as if it were going away from the paper towards the back of the page) with the narrow edge of the wedge coming from the center of the bond.
(a.)

(b.)

CHAPTER 3

DIMETHENAMID-P MOBILITY IN PINE BARK AND PINE BARK MEDIA MIXES[1]

[1] Williams, A.P., M.A. Czarnota. To be submitted to Hort Science.

Abstract

Soilless growing media dominates the containerized ornamental industry. Pine bark alone and in media mixes constitute most of the soilless media used in the containerized ornamental industry in the Southeast United States. With high organic matter content coupled with limited cation exchange capacity (CEC), surface area, and bulk density, herbicide weed control in soilless media can vary. Limited information exists about the interaction of soilless growing media with herbicides. Dimethenamid-P was recently registered by BASF for control of annual grasses, broadleaf weeds, and sedges in containerized ornamentals. A study was designed to examine dimethenamid-P movement in two soilless media: pine bark alone and a soilless mix containing pine bark. Collected leachate was analyzed using HPLC (high performance liquid chromatography). Studies indicated that no detectable leaching occurred in either of the soilless growing media, although leachate was detected in sand filled containers.

Significance to the Nursery Industry

Dimethenamid-P, [(S)-2-chloro-N-[(1-methyl-2-methoxy) ethyl]-N-(2,4-dimethyl-thien-3-yl)- acetamide] herbicide is used to control annual grasses, broadleaf weeds, and sedges in containerized ornamentals. Dimethenamid-P is applied alone or in combination with other herbicides as a spray, but is also available in a granular formulation with pendimethalin. Dimethenamid-P interactions with soil (Zimdahl and Clark, 1982; Mueller et al., 1999; Vasilakoglou et al., 2001; Bollman and Sprague, 2007; Bollman and Sprague, 2008) and hydroponic solutions (Osborne et al., 1995) have been researched. However, there is limited information concerning dimethenamid-P interactions with soilless growing media. The objective of this research was to determine the influence of two soilless media mixes on dimethenamid-P persistence and mobility.

Introduction

The potential of water contamination with herbicides is a continued concern. The USEPA, USDA, NAPIAP along with the agricultural chemical industry, evaluate herbicides prior to registration in order to assess persistence and potential water contamination. After evaluation, these organizations will collaborate in registering herbicides to ensure that it will not have unreasonable harmful effects on humans, the environment, and non-target species. In a nursery setting, ground and surface water contamination can occur from herbicides leaching from treated containers. Herbicides have the potential to leach through the growing media and eventually exit from the container, potentially causing unintended effects on the environment and if pond water is recycled, to the other crops in the nursery. Dimethenamid-P, the active isomer of dimethenamid, is a herbicide that has been recently introduced into the nursery industry. Previous studies have addressed dimethenamid-P and other chloroacetamide herbicide

interactions with soil (Zimdahl and Clark, 1982; Mueller et al.,1999; Vasilakoglou, 2001; Bollman and Sprague, 2007; Bollman and Sprague, 2008), but dimethenamid-P interactions with soilless growing media have not been reported.

Chloroacetamide herbicides are absorbed by roots, shoots, or cotyledons, and transported within the susceptible targeted plant via the xylem by acropetal movement, inhibiting early weed development (Böger et al., 2000). The dimethenamid-P molecule has a molecular weight of 275.79g/mole, a density at 1.19 g/ml (25 C) and water solubility of 1174 mg/L (25 C) (Senseman, 2007). Dimethenamid-P has no pK_a due to an inability to become ionic, and has a K_{ow} of 141(+/- 6) at 25 C (Senseman, 2007). Dimethenamid-P was reported to have an average half-life (DT_{50}) of 20 days in 10 field studies conducted in Europe and North America (Senseman, 2007).

Herbicides can become unavailable in soils or growing media due to adsorption (substituted ureas and chloroacetamides), plant uptake (all), volatilization (thiocarbamates), photochemical decomposition (dinitroanalines), runoff with treated soil (all), microbial breakdown (all), chemical breakdown, and leaching (imidazolinones) (Koren et al., 1969; Liu et. al, 1970; Zimdahl and Clark, 1982; Weber, 1990; Sorokina and Thomas, 1997). Many factors affect these processes including chemical family, pH, organic matter content of the soil or growing media, soil or substrate temperatures, and rainfall or irrigation volumes (Zimdahl and Clark, 1982; Beigel et al., 1999; Ma et al., 2006; Bollman and Sprague, 2007). Dimethenamid-P adsorption to a growing medium is particularly sensitive to components like organic matter and clay content (Bollman and Sprague, 2007). According to Senseman (2007), dimethenamid-P is moderately soil adsorbed, primarily degraded by microbes, and has low volatilization and photodegradation losses. In soil, most chloroacetamide herbicides are affected by rapid

microbial degradation, the half-life in the soil is generally short-lived (Zimdahl and Clark, 1982). The objective of this research was to determine the influence of two soilless media mixes on dimethenamid-P persistence and mobility.

Materials and Methods

Studies were conducted between January and September 2009. All experiments were conducted at the University of Georgia, Griffin Station, Griffin, GA.

Substrate Testing. In this study, two types of soilless growing media were used to test the leaching of dimethenamid-P; one being pine bark alone and the other being a commercial pine bark mix. A soil test conducted by the UGA Soil, Plant, and Water Laboratory was taken to evaluate the properties of the soilless growing media utilized in this study (Table 3.1). The bark used for both soilless growing media[1]were composed of mainly processed Loblolly pine (*Pinus taeda*) and Slash pine (*Pinus elliottii*). The commercial mix, known as Fafard® 52[1], contained Canadian sphagnum peat moss, vermiculite, perlite, starter nutrients, a wetting agent, and dolomitic limestone in addition to the pine bark. These elements mixed together form a soilless medium that is coarse, well drained, and has adequate air space.

The water holding capacity of each media was determined. Polyvinyl chloride (PVC) pipe[2] was cut into 4 identical cylinders and weighed. Rubber bands[3] and cut pieces of cheese cloth[4] were individually weighed. The two soilless media, pine bark and pine bark mix, were dried in a laboratory oven[5] at 72ºC for 48 hours. Two PVC cylinder units were filled with pine bark and two were filled with the pine bark mix. Both ends of each cylinder were then covered with cheese cloth held in place by the rubber bands. Each unit was then weighed. The weight of the pipe, cheese cloth, and rubber bands were subtracted from the total weight, giving the weight of the media in each unit. The media filled cylinders were then submerged in a container filled

with 7500 ml of distilled water for 72 hours. Using a clamping device, the cylinders were then suspended over a container to collect the water. The cylinders were monitored until they were at field capacity (no more water dripping from cylinder) and then reweighed. The wet cheese cloth and rubber bands were also weighed and subtracted from field capacity weight using the following formula:

water holding capacity= (wt. of wet media- wt. of dry media) ÷ wet volume of container

Calculations are shown in Table 3.2. The differences of the wet media weight and the dry media weight were calculated to obtain the water holding capacity for the two soilless media.

Lysimeter Installation, Settings, and Herbicide Application. Sixteen greenhouse benchtop lysimeters, identical to the lysimeters constructed by Grey et al. (2009), were used to determine dimethenamid-P movement in pine bark and the pine bark media mix (Figure 3.1). Pieces of PVC pipe 20 cm long of 30 cm wide were cut from a continuous section of a 600 cm pipe[6]. These segments, along with a PVC end cap[6], served as the base to contain the media. Prior to assembly, each piece of the PVC pipe and caps were cleaned with methyl-ethyl-ketone and an acetone-based cleaner[7] and dried. One of the end sections of the pipe and the inner lip of the cap were coated with an all purpose solvent weld[7]; after which, the two pieces were joined and sealed, forming an open top cylinder structure (Figure 3.1). Each of the PVC pipe and cap structures were held for approximately 2 minutes for the weld to set, and then dried for 2 days. A 2.5 cm hole was drilled in the bottom center (the middle of the cap) of the PVC lysimeter structures. A 34 mm wide x 55 mm long polypropylene funnel[4] was placed in the hole and glued in place with clear silicone[7] and allowed to dry for 24 hours. The funnels were loosely filled with glass wool[8]. An 18/14 mesh fiberglass screen[9] was cut to a 30 cm diameter, to fit the circumference of the unit, and was placed in the bottom of the lysimeter. Both the glass wool

and fiberglass screen served as barriers to keep the lysimeter unit from clogging. A foundation was built from two treated 5 cm thick x 10 cm wide x 2.4 m long treated boards that were screwed onto the bottom of 22.7 L buckets and was placed on a greenhouse bench. The lysimeters were placed onto this foundation, funnels facing down, between the two boards. Temperatures in the greenhouse ranged from 20 (±5) to 45 (±5) ºC for the extent of the study. Extreme fluctuations were due to two electricity outages, one in the month of March and the other in the month of July.

An irrigation system was constructed on the greenhouse bench that consisted of four risers containing 6 sprinkler heads[10] that delivered approximately 30 cm of water/ hour. Prior to treatment, all lysimeters were washed with potable water to ensure the lysimeter system was flowing properly. Once the lysimeters were washed, they were uniformly filled with 1.3 cm layer of sand[11] on top of the fiberglass screen. Four of the lysimeters contained sand only. The remaining 12 lysimeters were filled with an additional 15.2 cm layer of either pine bark or pine bark media mix, similar to a standard 3.8 L container. Media was prepared for the study by leaching the media with a 2.5 cm irrigation event. Leaching occurred daily for seven consecutive days to ensure the lysimeters flowed freely. Each treatment (pine bark and pine bark media mix) had three treated replications and three nontreated (NTC) controls. The sand treatment had only two treated and one NTC. The replications and NTC were arranged in a randomized design supported by the foundation risers on the greenhouse bench. Each lysimeter unit was labeled[12]. Prior to application of the herbicide containing dimethenamid-P, the sprinklers were turned on until a beaker, appropriately labeled to match the corresponding unit, placed under the lysimeter was filled with 2.5 cm of water (approximately 5 minutes). Two 1.5 ml samples were then taken from the collected leachate of each lysimeter using a pippette[13]. All

samples were saved in sealable 2 ml black storage vials[14] (to prevent photodegradation), labeled, and then placed in a freezer at -10º C to maintain the integrity of the sample during storage.

Herbicide Application. Herbicide application was conducted outside of the greenhouse in a measured 3.66m x 1.83m block. Conditions outside during spraying were 14º C, 83% humidity, 3.0 kph with gusts up to 8.5 kph, pine bark temperature 15.5º C, and pine bark mix media14.5º C. The lysimeters to be treated were removed from the foundation risers and placed on concrete blocks, careful to keep the funnels from contacting the ground. Using a laboratory pipette, 25 mL of the herbicide[15] containing dimethenamid-P was drawn and added to 3 L spray bottle filled with 2 L of deionized water. The formulated herbicide contained 718.98 g of active ingredient (dimethenamid-P) per liter. To apply the herbicide, a CO_2 backpack sprayer[16] calibrated to deliver 182 L/ha was used. The spray boom was equipped with 8003VS spray tips and 50 mesh screens[17]. All spray equipment was re-calibrated for accuracy before applications were made, and protective clothing and eyewear were worn as recommended by the label. The rate of dimethenamid-P applied to lysimeters in the spray area was 1.68 kg a.i./ha. The entire capturing surface area of the lysimeter (pipe and cap) was calculated to be 2,591.65 cm^2. To get the approximate amount of applied herbicide to each lysimeter the following formula was used:

$$(0.259165\ m^2 * 1.68\ kg\ a.i./ha) \div (10{,}000\ m^2 * x)$$

$$x = 0.00004353972\ kg\ a.i \text{ or } 43539.72\ \mu g\ a.i./surface\ of\ each\ lysimeter$$

After the lysimeters were treated, care was taken to place the units back to their appropriate positions on the supports. Once back in position, the overhead sprinklers were activated for 5 minutes to simulate 2.5 cm watering event and to activate the herbicide. This leachate was collected and saved to be analyzed later.

Leachate Collection. Leachate from the lysimeters was collected for 2 hours. Once gravitational water flow stopped into beakers under the lysimeters, two 1.5 mL representative samples from each lysimeter unit were collected and stored. Volume of each beaker was also recorded. This process of taking duplicate samples and recording leachate volume was repeated once daily for the first week , then every other day for 60 days, then every fourth day for 53 days, and then once a week for 3 weeks totaling a combined time of 3 months and 4 days. Days that samples were not taken, sprinklers were still run the allotted 5 minutes, but the leachate was not collected during these off days. This irrigation process was continued to keep the media at full water holding capacity.

A separate leachate analysis of just the label rate of dimethenamid-P and no soilless media content, other than the materials used in the construction of the lysimeters, was conducted to ensure that the herbicide was not bound to extraneous materials (e.g. glass wool, PVC, etc). Leachate was collected and analyzed by HPLC.

Sample Preparation and HPLC Analysis. Prior to analysis, stored leachate samples were removed from the freezer and thawed in the lab till they reached room temperature. Next they were filtered using a 5 mL slip tip syringe[18] and a syringe tip disposable filter with a pore size of 0.45 µm and 13 mm diameter[19]. These samples were filtered directly into amber glass 1.8 ml injection vials[20], sealed with a screw septa cap, and labeled[12]. Amber glass was used to help provide UV light protection for the samples. Those samples which were anticipated to be below the detection limits of dimethenamid-P, were combined and dried with a RE-111 Büchi rotovap[21], and further evaporated to eliminate all water by using a Pierce Reacto-therm. These samples were resolubilized with 500 µL of acetonitrile (ACN), and then mixed, filtered, and transferred to a 1.8 mL injection vial. Combining samples, evaporating, and resolubilizing,

allowed for approximately a 6 times more concentrated sample. Leachate samples were analyzed for dimethenamid-P using a HPLC (high performance liquid chromatography) system[22]. The column used for HPLC analysis was a Sonoma C18, 15 µ, 100 Å, 25cm x 4.6mm[23]. Settings for sample analysis were isocratic using a mixed mobile phase containing 70% HPLC grade ACN and 30% HPLC grade water. Before and after sample analysis, the column was cleansed with 100% ACN for 1 hour to ensure any contamination was cleansed from of the column. Retention time was approximately 7 minutes and peaks were monitored at a wavelength of 238 nm.

A standard curve was developed to verify the concentration of dimethenamid-P. The standard curve was created by diluting technical-grade dimethenamid-P[15] ranging from 2,758 µg/L- 27,579,000 µg/L. The 27,579,000 µg/L solute - ion was diluted by a factor of ten 4 times to get 2,758 µg/L. The concentrations are demonstrated in Figure 3.2. A sample from the developed standard curve was also run prior to samples, ensuring that the dimethenamid-P was traceable and the machine was working properly.

Statistical Analysis

Appropriate models and graphs were completed using Microsoft Excel 2007®. For linear regression the following equation was utilized:

$$y = mx + b$$

where m determines the slope and b determines the point where the line crosses the y axis.

Results and Discussion

Through HPLC analysis, it was determined that dimethenamid-P was not detectable from either the pine bark or the commercial pine bark mix soilless media throughout the course of the four and a half month leachate collection. However, herbicide was detected in samples from the sand leachate and leachate with no media (Figures 3.3 and 3.4, respectively). Concentration of the herbicide in the leachate dropped between initial collection (leachate collected within first 2 hours of spraying) and day 2, approximately 10 fold for sand and 5 fold for no media. Table 3.3 compares the leachate recovery rates of dimethenamid-P in the lysimeters filled with sand and no media to the expected amount of applied dimethenamid-P to each lysimeter, which was 43,540 µg of dimethenamid-P. The leachate recovery from the lysimeters filled with sand after 2 days was 46 %. After 3 days no dimethenamid-P was detected. Leachate recovery from the no media lysimeters was 61% after 6 days. After 5 days, concentrations of dimethenamid-P fell to nearly equal levels of recovery in the lysimeters with no media (Figure 3.4). As for the fate of the unrecovered dimethenamid-P in the lysimeters filled with sand, dimethenamid-P could have been bound to the sand surface, leached at levels not detectable by HPLC analysis, or lost by application error (drift, volatilization, etc.). Also, over estimation of the theoretical lysimeters captured amount could have occurred. As for the unrecovered dimethenamid-P in the lysimeters without media, the amount of dimethenamid-P leachate after 7 days was not included.

The fate of dimethenamid-P in the lysimeters filled with soilless media could be contributed to adsorption to the media, degradation, and/or volatility. As with most preemergence herbicides, adsorption of the herbicide to the growing media accounts for the weed control of the preemergence herbicide. Simultaneous bioassay studies revealed that

dimethenamid-P provided weed control for up to 15 weeks, meaning that this herbicide is available for uptake in the upper portion of the growing media.

Although the herbicide was available for uptake by the weed species, the fate of dimethenamid-P could have been contributed by other causes. The dimethenamid-P molecule is relatively simple in terms of herbicide structure (i.e. no benzene rings), thus making it easier to be broken down or degraded by microorgranisms as a carbon source. A study of three acetanilide herbicides showed that degradation rates increased with increasing soil moisture content and temperature (Zimdahl and Clark, 1982). According to Senseman (2007), two of the most important factors affecting the dissipation of chloroacetamide herbicides are adsorption and microbial decomposition. Moreover, the growing media utilized in this experiment was composed mainly of organic matter (over 60%) it could be hypothesized that the herbicide was adsorbed to the organic components of the growing media and was not made available to leach. However, because so much of the herbicide was lost within hours and days of the application, it would be difficult to believe that the dimethenamid-P fate was lost to microbial degradation.

Another possibility of herbicide loss could be contributed to volatility. According to Senseman (2007), dimethenamid has low losses in soil due to volatility, however, it is possible that some of the herbicide could have evaporated, volatilized, or simply did not make it onto the lysimeter unit due to drift when it was sprayed outside. The rate and amount of a herbicide volatilizing is dependent on factors such as temperature, soil water content, water vapor pressure immediate to the soil-plant-air boundry, solar radiation, and the speed of wind (Prueger and Pfeiffer, 1994). According to studies by Hargrove and Merkle (1971), alachlor losses due to volatilization increased with increasing relative humidity. In another study, around 50% of alachlor and metolachlor volatilized from a glass surface 8 days after application, while only

0.1% was lost from the soil surface (Parochetti, 1978). In metolachlor studies, Prueger and Pfeiffer (1994) found that despite possessing a fairly low vapor pressure (1.3×10^{-5} mm of Hg), the herbicide was relatively volatile (79% volatilized from the glass in the research). A CDAA [(2-chloro-N, N-diallylacetamide)] study by Deming (1963) revealed that relationships between volatility and temperature were strongly influenced by the amount of water speeding up CDDA volatility loss.

Conclusions

Results from this study demonstrated that through HPLC analysis dimethenamid-P was recovered at 46% and 61% from the lysimeters containing sand and those with no media, respectively. According to the data much of the dimethenamid-P pulsed out of these 2 lysimeter profiles during the first few hours after application, and revealed that the construction materials and sand were possibly preventing some of the dimethenamid-P from leaching. However, the dimethenamid-P was not detected in the leachate from the pine bark and pine bark mix at any point during the study. The major point of loss, in the lysimeters filled with soilless media, is likely to the absorption / adsorption of dimethenamid-P to the growing media, making it possible for dimethenamid-P to provide weed control of common lambsquarters (*Chenopodium album*) and tall fescue (*Festuca arundinacea*) in a simultaneous study for up to 15 weeks. However, that does not mean other mechanisms of loss were not involved. Other avenues of loss could include volatilization, chemical breakdown, and/or microbial breakdown. Although speculative, if one compared the fate of the other acetamide herbicides, a likely fate of the herbicide was microbial breakdown. However, it is unlikely that microbes would have broken down dimethenamid-P immediately after application, and no dimethenamid-P was ever recovered. What seems most

likely is that soilless growing media bound the majority amounts of dimethenamid-P. However, limited scientific information is available to defend or refute this information.

Table 3.1. Soil test conducted by the University of Georgia Soil, Plant, and Water Laboratory located in Athens, GA 30605.

Media	LBC[1] (ppm $CaCO_3$/pH)	pH[2]	OM[3]
Pine Bark	3472	3.96	69.88
Pine Bark Mix	1150	5.49	61.6
Sand	39	5.27	0.02

[1]Measurement of lime buffer capacity
[2]Soil pH and salt concentration
[3]Organic matter is determined by the "loss of ignition" method for 3 hours at 360º C

Table 3.2. Water holding capacity calculations.

Media	Media Wet Wt.- Media Dry Wt. ÷ Total Volume of Container	% Water Holding Capacity
Pine Bark	(265.35g-121.96g) ÷ 606.42g	24%
Bark Mix	(389.35g- 119.55g) ÷ 730.5g	37%

Figure 3.1. Lysimeter units were constructed with PVC pipe/cap resting on risers created from treated boards and buckets on a greenhouse bench. A hole was drilled in the center of the cap and a small funnel was placed at the bottom of the lysimeters to allow leaching to be collected in beakers (not pictured). Entire system was irrigated with overhead sprinklers. All lysimeter data was collected in the greenhouse.

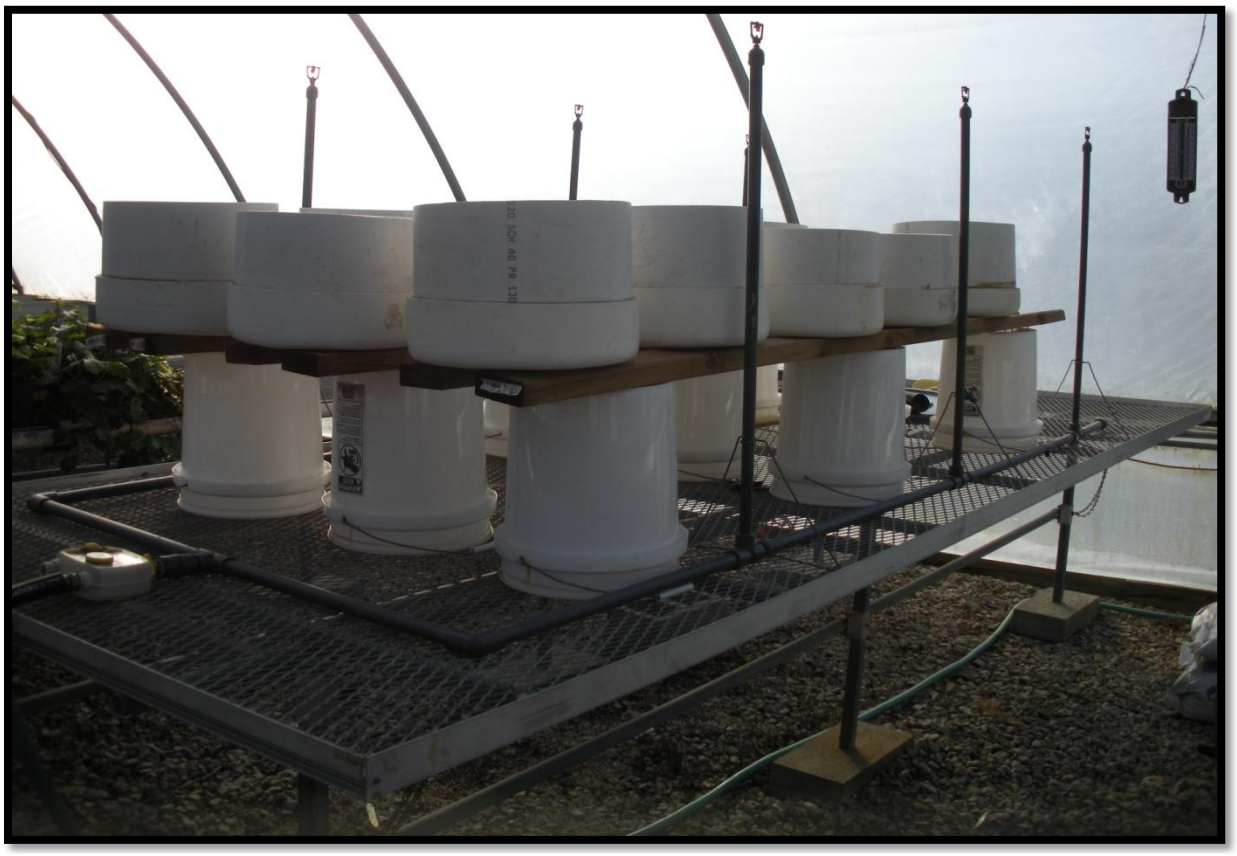


Table 3.3. Dimethenamid-P leachate recovery comparing expected and actual leachate values collected.

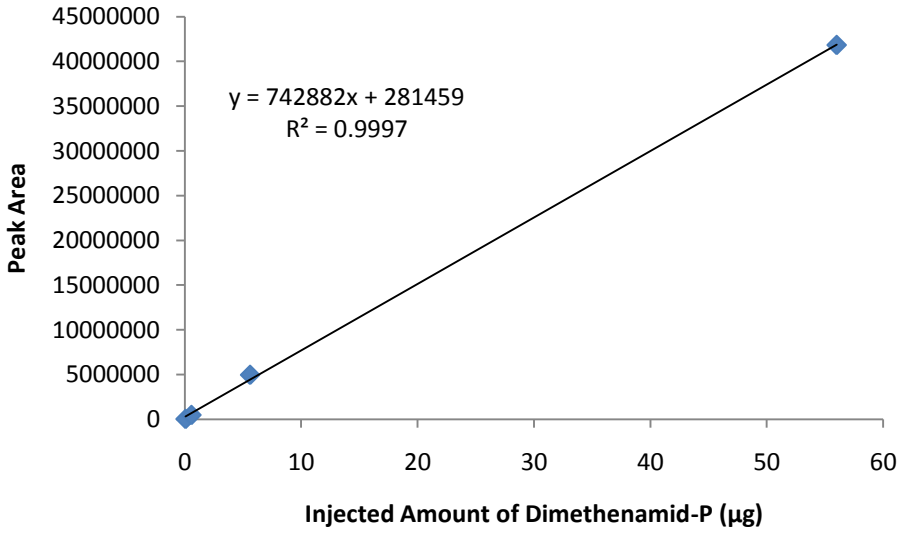
Lysimeter	Average peak area	Average collection volume (µl)	Average Amount D-P[2] injected in HPLC (µg/20µl)	Average amount D-P collected in beaker (µg/ collection)
Sand[1]				
1st collection	741,917	320,000	0.85	13,600
2nd collection	314,218	316,000	0.36	5,688
3rd collection	51,493	271,000	0.06	813
Total				20,101
% Recovery				46

No Media[1]				
1st collection	730,310	363,000	0.83	15,065
2nd collection	133,453	371,000	0.15	2,783
3rd collection	137,644	452,000	0.15	3,390
4th collection	165,692	442,000	0.19	4,199
5th collection	90,199	411,000	0.10	451
6th collection	83,692	373,000	0.10	419
7th collection	80,524	401,000	0.09	363
Total				26,670
% Recovery				61

[1]The approximate amount of dimethenamid-P applied to surface area of lysimeters was 43,540 µg a.i. with a predicted peak area of 460,582.

[2]D-P is abbreviated for dimethenamid-P.

Figure 3.2. Standard Concentrations Used for HPLC Analysis of Dimethenamid-P[1] leachate



[1]The formulated dimethenamid-P herbicide projected a peak area of 1914160.

Figure 3.3. Amount of Dimethenamid-P leachate collected from treated sand replications.

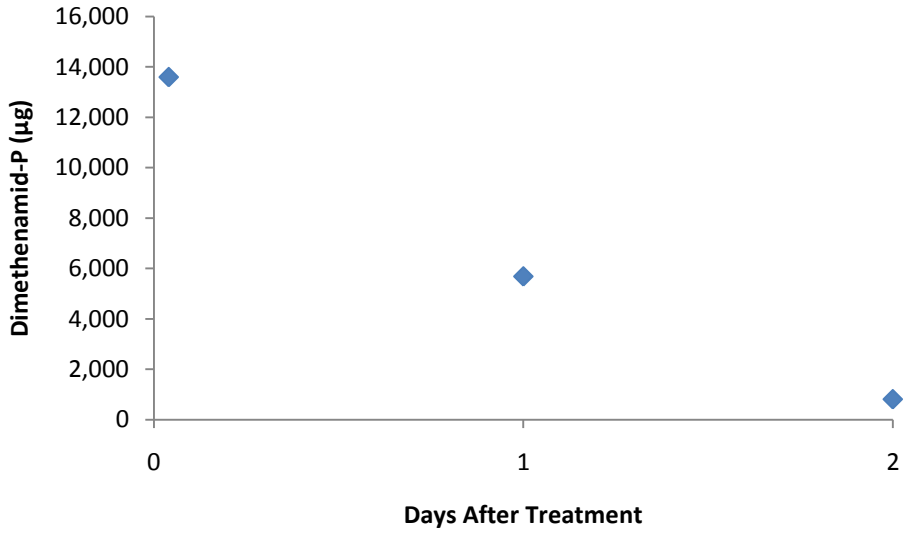
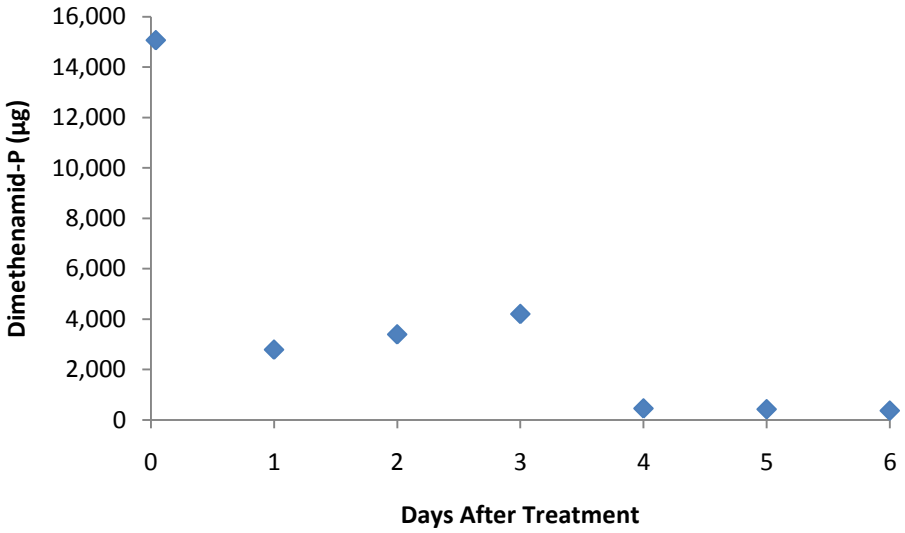


Figure 3.4. Amount of Dimethenamid-P leachate collected from treated lysimeters replications containing no media.



CHAPTER 4

ASSESSMENT OF DIMETHENAMID-P HERBICIDE ACTIVITY IN PINE BARK MEDIA UTILIZING TWO INDICATOR SPECIES[2]

[2] Williams, A.P., M.A. Czarnota. To be submitted to Hort Science.

Abstract

Pine bark and mixes containing pine bark encompass much of the soilless media used in the containerized ornamental industry. Limited information is available concerning the interaction of soilless growing media and herbicides. Dimethenamid-P has recently been labeled for control of weeds in the ornamental industry. A study was designed to determine the influence of pine bark based media on the activity of dimethenamid-P using the two indicator species common lambsquarters (*Chenopodium album*) and tall fescue (*Festuca arundinacea*). It was found that dimethenamid-P was able to provide significant control of tall fescue in pine bark mix for 15 weeks after treatment. In addition, a dose response assay was conducted to determine the extent of control: 0.21 kg a.i./ha, 0.42 kg a.i./ha, 0.84 kg a.i./ha, 1.68 kg a.i./ha, 3.36 kg a.i./ha, 6.72 kg a.i./ha, 13.44 a.i./ha, with 1.68 kg a.i./ha being the recommended labeled rate. Data indicated that dimethenamid-P provided substantial control of common lambsquarters and tall fescue at all rates compared to the nontreated plantings.

Introduction

Weeds infest containers in commercial ornamental nurseries via wind and irrigation (Horowitz and Elmore, 1991), through unsanitary equipment, animal distribution, and by human traffic from contaminated clothing and shoes (Stamps, 1997). Weeds compete for water, nutrients, light, container space, and may increase the chances for insects, diseases, and vertebrate pests to populate (Altland, 2003; Wallace and Hodges, 2007). In the container nursery industry, it is most beneficial for weed germination to be prevented in order to have a successful nursery crop with optimum growth and development (Altland, 2003).

Dimethenamid-P, [(*S*)-2-chloro-*N*-[(1-methyl-2-methoxy) ethyl]-*N*-(2,4-dimethyl-thien-3-yl)- acetamide] is a preemergence herbicide that has been recently labeled for the container ornamental industry. Some important nursery weeds that dimethenamid-P control include: annual bluegrass (*Poa annua*), large crabgrass (*Digitaria sanguinalis*), barnyard grass (*Echinochloa crus-galli*), fall panicum (*Panicum dichotomiflorum*), goosegrass (*Eleusine indica*), carpetweed (*Mollugo verticillata*) common groundsel (*Senecio vulgaris*), spotted spurge (*Euphorbia maculata*), bittercress (*Caramine spp*.), liverwort (*Marchatla polymorpha*), and yellow nutsedge (*Cyperus esculentus*).

Dimethenamid-P has a molecular weight of 275.79g, a density of 1.19 g/ml (25C) and is water soluble at 1174 mg/L (25 C) (Senseman, 2007). $C_{12}H_{18}ClNO_2S$ is the molecular formula for dimethenamid-P. It has no pK_a since it is non ionic, and has a K_{ow} of 141+/- 6 at 25 C (Senseman, 2007). Dimethenamid-P is dark in appearance and has a noticeable odor similar to tar. At 55 C, dimethenamid-P was stable for 90 days (Senseman, 2007). In soils, the approximate half life of dimethenamid-P has ranged from 1-2 weeks in the southern region of the United States to 5-6 weeks in the northern part of the U.S. (Senseman, 2007). Its vapor pressure

was measured at 3.68 x 10^{-2} Pa (25 C) and the herbicide has reportedly low losses in the soil due to volatility (Senseman, 2007).

In modern literature, dimethenamid-P is classified as a chloroacetamide herbicide. This class of herbicides, although not completely understood, are thought to have a mechanism of action which is a strong inhibition of very long chain fatty acid synthesis (Senseman 2007). According to previous research, chloroacetamide herbicides are absorbed primarily through shoots in grasses (Bollman et al., 2008) , by the roots in broadleaf plants, (Le Baron et al., 1988), or cotyledons as they develop through treated soil (Böger et al., 2000; McGregor et al., 2005). When chloroacetamide herbicides are taken up by the roots, shoots, or cotyledons, they are transported within the susceptible targeted plant via the xylem by acropetal movement, inhibiting early weed development (Böger et al., 2000). Even though weed seeds typically germinate, dimethenmid-P affects cell division (cytokinesis) thereby blocking seedling growth and the affected plant remains stunted and distorted, usually unable to emerge above the soil (Fuerst, 1987; Böger et al., 2000; McGregor et al., 2005).

Application and use of dimethenamid-P has increased in the past decade (Anderson et al., 2005), fueling interest in the level of potency and effects of dimethenamid-P for the ornamental industry on weeds in common soilless container substrates. Determination of associations among herbicide dose and plant response is essential in understanding efficacy and mode of action for a particular herbicide (Seefeldt et al., 1995), hence the need to conduct a bioassay, or a dose response study, for experiments involving herbicide application associated plant responses.

The objectives of this research were to: a) determine herbicidal control that dimethenamid-P demonstrates on the indicator species, common lambsquarters and tall fescue, in pine bark and a commercial pine bark mix media in a container repeated bioassay and b) assess

the potency of dimethenamid-P by analyzing the reaction that follows application of various rates on common lambsquarters and tall fescue in pine bark and a commercial pine bark mix media substrate.

Materials and Methods

Studies were conducted between January and September 2009. All experiments were conducted at the University of Georgia, Griffin Campus Griffin, GA. This research was conducted in a greenhouse with temperatures that ranged from 20 (±5) to 45 (±5) ºC for the extent of the study. Extreme fluctuations were due to two electricity outages, one in the month of March and the other in the month of July.

Indicator Species. For this study, common lambsquarters (*Chenopodium album*) and tall fescue (*Festuca arundinacea*) were chosen for indicator species because of their reliable germination and sensitivity to dimethenamid-P. Lambsquarters and tall fescue respectively exhibited a 30% and 45% germination rate in nontreated media. Other species that were considered unreliable (<10% germination rate in nontreated media) for these studies were *Euphorbia prostrata*, *Digitaria sanguinalis*, *Fatoua villosa*, *Ipomoea tricolor*, *Phyllanthus tenellus*, and *Triticum aestivum*, and therefore were not used in these studies. On the opposite end of the spectrum, *Avena sativa* was considered as an indicator species, but was found to have an inadequate response to dimethenamid-P.

Common lambsquarters, also known as pigweed, meal weed, or white goosefoot, belongs in the goosefoot or Chenopodiaceae family and is native to Eurasia (Uva et al., 1997; Bryson and Delfelice, 2009). Seeds of this plant are lens-shaped, black, and shiny (Bryson and Delfelice, 2009). Common lambsquarters is a branching broadleaf plant that can range from 0.3-1.8 meters tall, with long, triangular coarsely toothed shaped alternate leaves (hence the name,

goosefoot) that are powdery gray underneath. Stems are hairless, have vertical ridges, commonly with strips of maroon color. Flowers, produced from June to September, are clustered paniculate spikes with a green calyx and no petals and fruits are utricle with a star-shaped calyx of 5 sepals virtually encasing the seed (Uva et al., 1997; Bryson and Delfelice, 2009).

Tall fescue or meadow fescue, is a member of the Poaceae (grass) family, and is native to Europe. It is an erect, tufted perennial grass that can reach heights of up to 1.4 m. Tall fescue produces short rhizomes but has a clustered growth habit and spreads mainly by erect tillers. Mature plants have leaves that are long, flat, and sheaths that can range from smooth to major ciliated auricles. Flowers are panicle, varying from 10-30 cm long, narrow, with short pediceled spikelets; fruits are caryopsis (Bryson and Delfelice, 2009).

Repeated Bioassay. A repeated bioassay using common lambsquarters [24] and tall fescue was conducted to determine the efficacy rate of the dimethenamid-P herbicide. Plantings were arranged by species and treatment with 5 replications. Eighty 3.8 L containers filled with pine bark[1] and eighty 3.8 L containers filled with a commercial pine bark mix media[1] were sprayed at the recommended label rate of 1.68 kg a.i./ha. The herbicide[15] contains 718.98 grams of active ingredient (dimethenamid-P) per liter. The media used for these studies was composed of bark, from predominately processed Loblolly pines (*Pinus taeda*) and Slash pines (*Pinus elliottii*). The commercial mix, Fafard® 52, contained Canadian sphagnum peat moss, vermiculite, perlite, starter nutrients, a wetting agent, and dolomitic limestone in addition to the pine bark.

A CO_2 backpack sprayer[16] calibrated to deliver 31.5 L/ha was used to treat the pots. The spray boom was equipped with 8003 VS flat fan spray tips which were filtered with 50 mesh screens[17]. All spray equipment was calibrated before applications were made, and protective

clothing and eyewear were worn. Environmental conditions at the time of application were 14º C, 83% humidity, 3 kph with gusts up to 8.5 kph, pine bark temperature 15.5º C and pine bark mix media at 14.5º C. Eighty containers each of pine bark and pine bark mix media were set aside in the greenhouse to serve as nontreated controls.

Prior to spraying, a set of treated and nontreated containers were seeded with common lambsquarters and tall fescue. Every 21 days, a set of containers were seeded, and seeding was repeated at 21, 42, 63, 81, 102, and 123 days. Weed counts of the two plant species were recorded every 21 days, after which, plant material was harvested to determine both fresh and dry weights. Harvested plants were dried using a laboratory oven[5] maintained at 70º C for 48 hours.

Dose Response Study. A biological assay, or dose response test, was conducted to help formulate an analytical response curve to dimethenamid-P. Likewise, lambsquarters and tall fescue were used in this study as indicator species. Treatments for the dose response included pine bark planted with lambsquarters, pine bark planted with tall fescue, pine bark mix media planted with lambsquarters, and pine bark mix media planted with tall fescue all of which had five replications for each rate. For each container, 20 seeds of the desired plant were counted and sown into the potting media. Herbicide was then applied at seven rates: 0.21 kg a.i./ha, 0.42 kg a.i./ha, 0.84 kg a.i./ha, 1.68 kg a.i./ha, 3.36 kg a.i./ha, 6.72 kg a.i./ha, 13.44 kg a.i./ha, with 1.68kg a.i./ha being the maximum labeled rate. For a fresh and dry weight comparison and plant counts, nontreated controls (NTC) of both common lambsquarters and tall fescue were grown in both pine bark and the pine bark mix media. Plant roots and shoots were harvested by removing the mass from the media and gently washing with deionized water and then blotting with a paper towel before weighing. The plants were grown for six weeks before harvesting.

Statistical Analysis. Seedling growth was determined by exhuming the plants from both treated and untreated pots, and determining plant fresh and dry weight. Appropriate models and graphs were completed using SigmaPlot® and Microsoft Excel 2007®. To show the relationships between the treated and nontreated plants, a bar graph using standard error (SE) bars was utilized in the repeated bioassay. For the dose response study, a negative exponential function was utilized on the data using the equation, $y = \beta_0 e^{-\beta 1x}$.

Results and Discussion

Those weeks that both the nontreated control and the treated replications did not grow as expected were due to two electricity outages, one in the month of March and the other in the month of July (Figures 4.1 and 4.2).

Repeated Bioassay. Plants grown in pine bark alone had overall poorer growth than plants grown in the pine bark mix media. Pine bark has less ability to retain water and nutrients when compared to the other soilless mix used in this study (Fafard® 52) due to added components. Nurserymen who use pine bark as a single component substrate have found that the material is too coarse in texture and may possess air pockets or not enough moisture available for newly formed roots (Bilderback and Lorscheider, 1994).

Although not statistically different, there was a substantial weight difference between the dry weights of the treated and nontreated tall fescue grown in the pine bark media mix at weeks 3, 6, 9, and 15 (Figure 4.1). Also, there were no statistically differences between Lambsquarters grown in pine bark and pine bark mix, although, weight differences were substantial (Figure 4.2). There were also some significant interactions between treated and nontreated plant counts (Table 4.1) using Fisher's LSD Test (α at 0.05). Tall fescue grown in pine bark mix showed significant

interactions between treated and nontreated plant counts at weeks 3-15 and for tall fescue grown in pine bark for weeks 3, 6, and 12. There were also significant interactions for lambsquarters grown in pine bark mix at weeks 6, 15, and 21and for lambsquarters grown in solely pine bark for weeks 3 and 6. Overall, dimethenamid-P was able to control the plants grown in pine bark mix longer than the plants grown in just pine bark.

Dose Response Study. Dry weights and counts for all plants grown in pine bark or pine bark media mix were compared at all dose response rates ranging from 0 to 13.44 kg a.i./ha. There were substantial differences between all treated and untreated plants (Figures 4.4 and 4.5), with the dry weight of the untreated plants being higher than the treated plants. However, because of plants growing in response to herbicide application, an expected linear response was not produced. Previous studies have shown that at low doses, herbicides can cause a general stress response and therefore grow more, even when a small dose of herbicide is applied, which may explain why dimethenamid-P triggered the plants to grow more at certain doses (Cedergreen, 2008). Germination percentages of the treated and nontreated plants and media are presented in Figure 4.5. As with the plant back, in the dose response studies, there was a substantial difference between plant growth from the pine bark and the pine bark mix.

Conclusions

Dimethenamid-P was able to significantly control tall fescue in pine bark mix for approximately 15 weeks in the repeated bioassay. The reason dimethenamid-P provided inconsistent control of the other plant and media combinations can only be speculated. As with most preemergence herbicides, adsorption of the herbicide to the growing media accounts for the weed control of the preemergence herbicide. In addition to adsorption, the fate of dimethenamid-P in the soilless media could be contributed to degradation and/or volatility.

As for the possibility of degrading, the dimethenamid-P molecule is relatively simple in terms of herbicide structure (i.e. no benzene rings), thus making it easier to be broken down or degraded by microorganisms as a carbon source. Most microbial breakdown processes however occur in a timed response, are not immediate, and depend on factors of the microclimate (Kaufman, 1966).

Dimethenamid-P has low losses in soil due to low volatility (Senseman, 2007). However it is possible that some of the herbicide could have evaporated or volatilized immediately after being applied. The rate and amount of a herbicide volatilizing is dependent on factors such as temperature, soil water content, water vapor pressure immediate to the soil-plant-air boundry, solar radiation, and the speed of wind (Prueger and Pfeiffer, 1994).

Figure 4.1. Growth response of *F. arundinacea* in treated and nontreated (A) pine bark mix and (B) pine bark. Data points are the means of five replications with the bars indicating the SE of the mean.

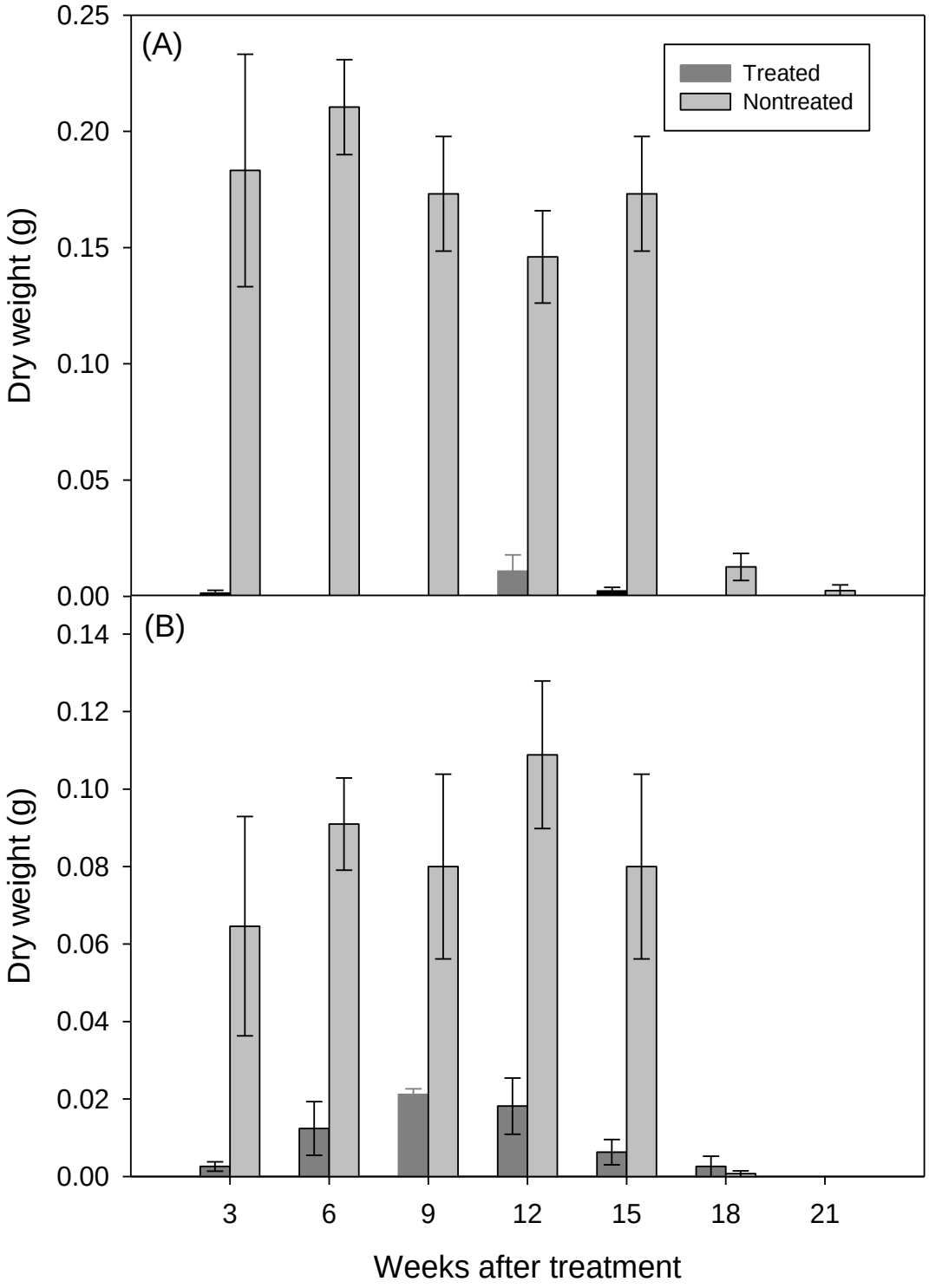


Figure 4.2. Growth response of *C.album* in treated and untreated (A) pine bark and (B) pine bark mix. Data points are the means of five replications with the bars indicating the SE of the mean.

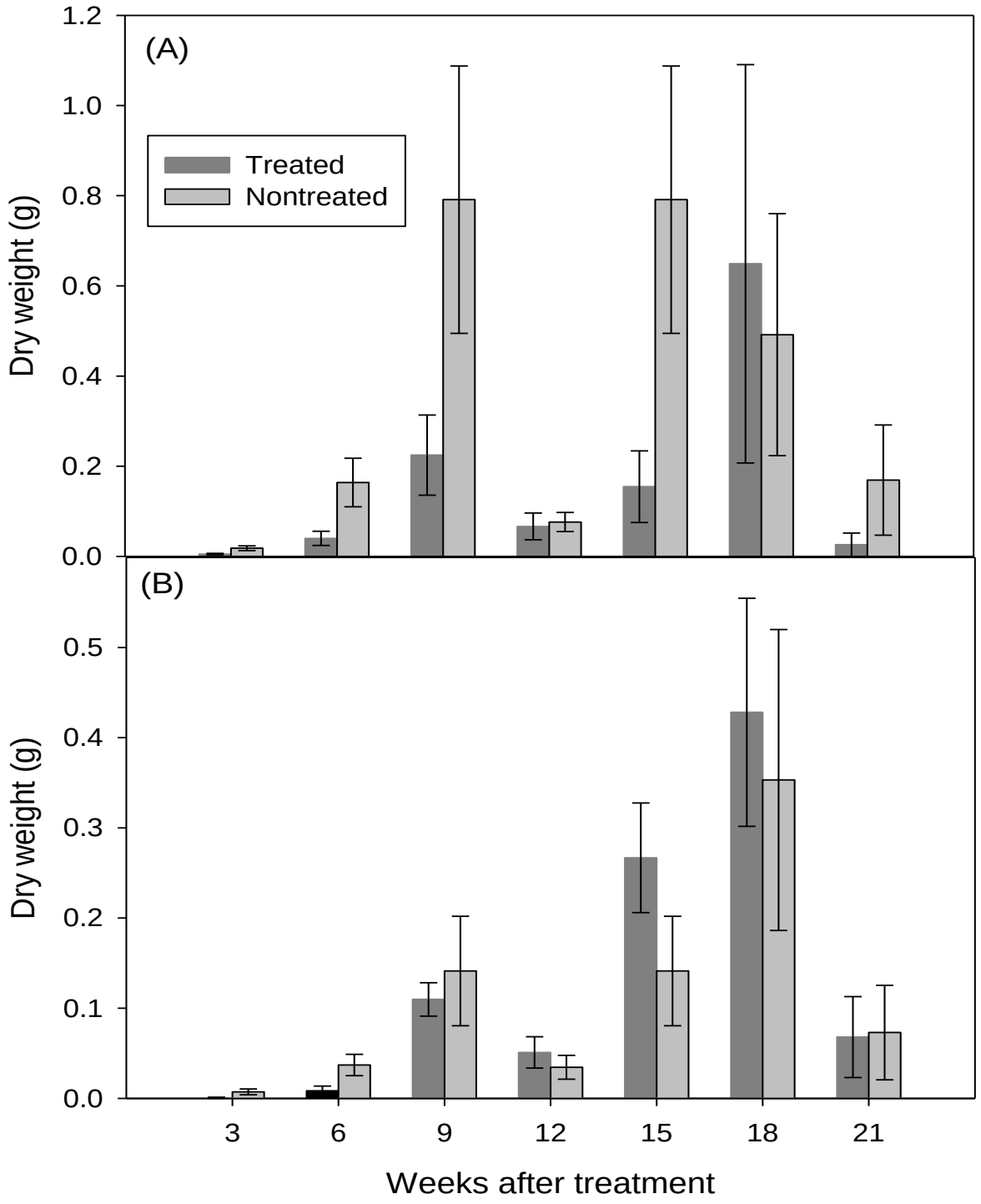


Table 4.1. Plant Back Germination. Significance values were calculated using Fisher's LSD test at α = 05.

Week	*F. arundinacea* in Pine Bark Mix	*F. arundinacea* in Pine Bark	*C. album* in Pine Bark Mix	*C. album* in Pine Bark
3	**	*	NS	*
6	***	**	*	***
9	**	NS	NS	NS
12	*	*	NS	NS
15	**	NS	**	NS
18	NS	NS	NS	NS
21	NS	NS	*	NS

* P < 0.05

** P < 0.01

*** P < 0.001

NS = non-significant

Figure 4.3. Plant dry weight of *C. album* and *F. arundinacea* grown in pine bark mix treated with various rates of dimethenamid-P.

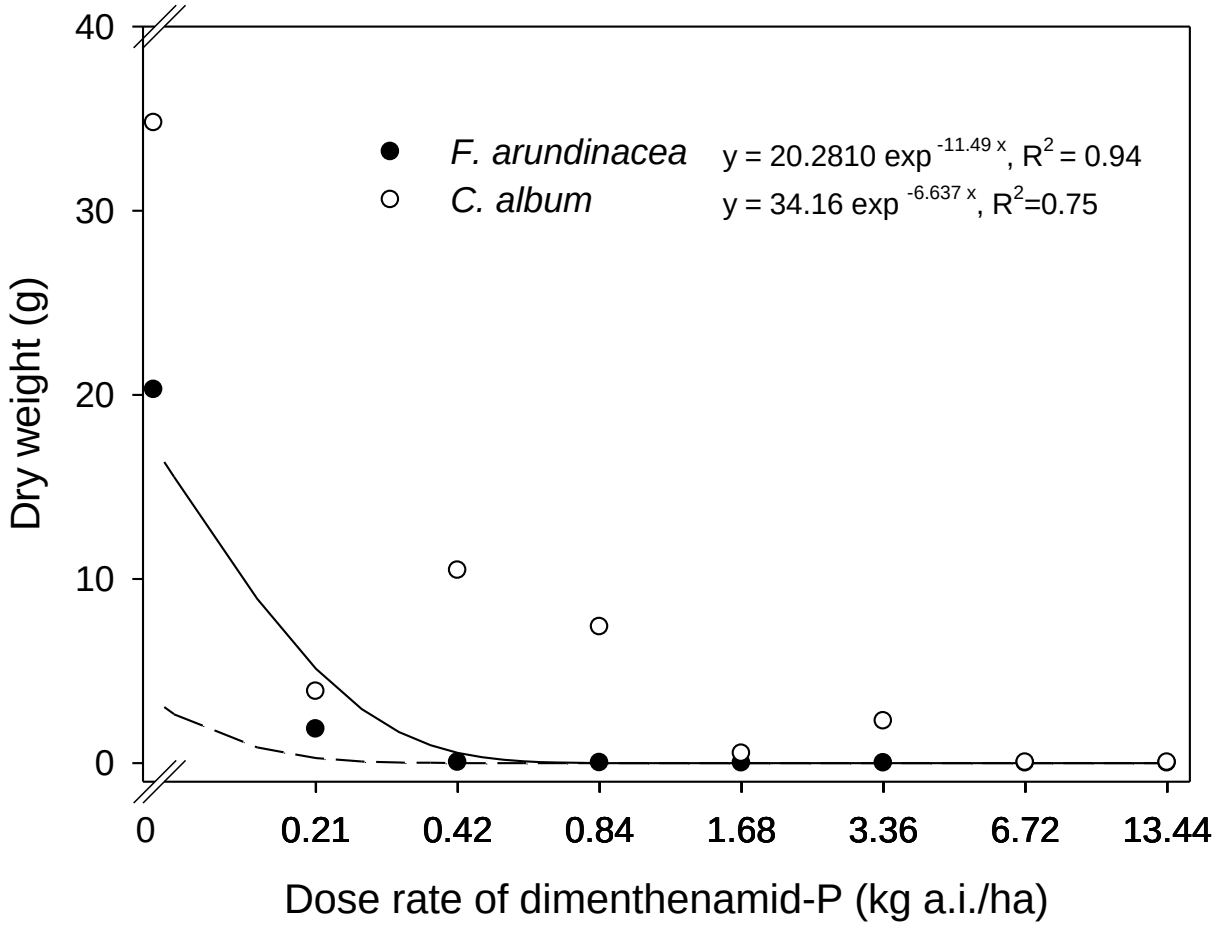


Figure 4.4. Plant dry weight of *C. album* and *F. arundinacea* grown in solely pine bark treated with various rates of dimethenamid-P.

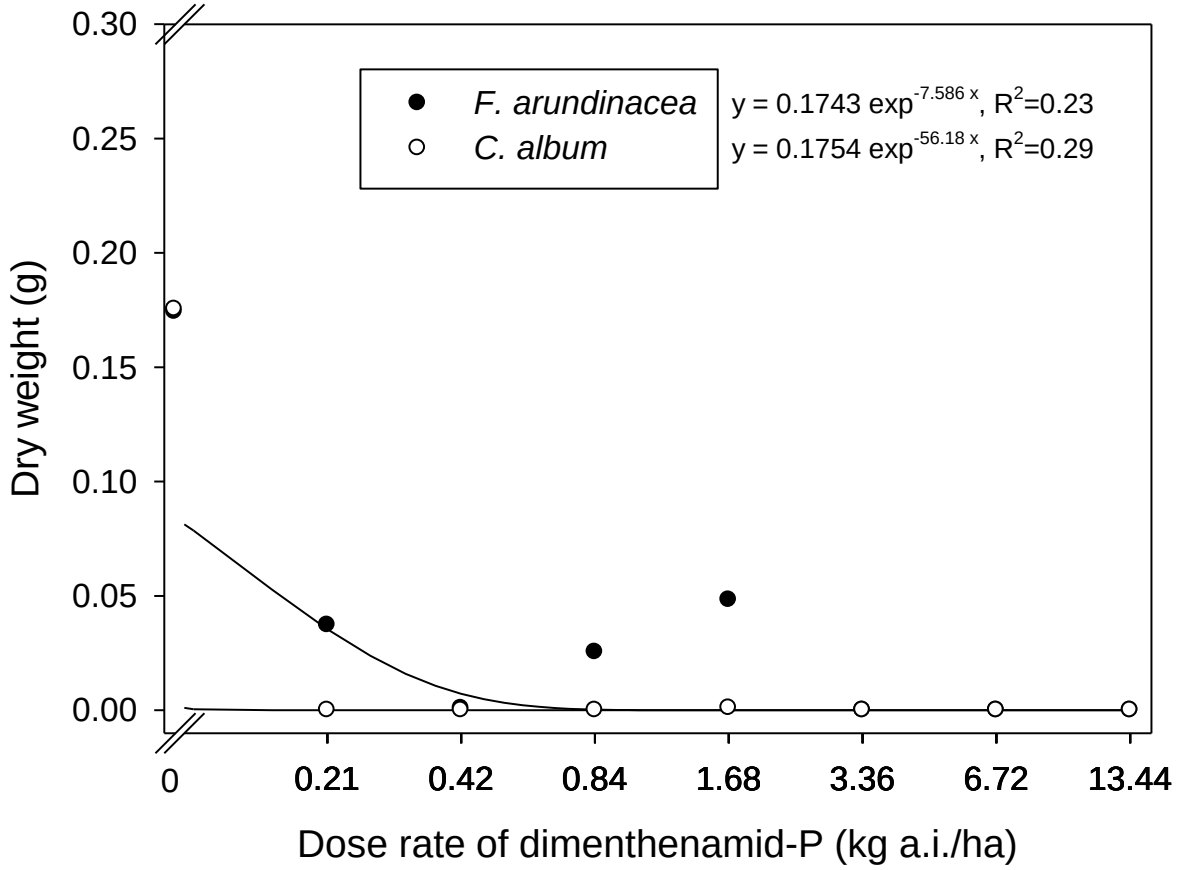
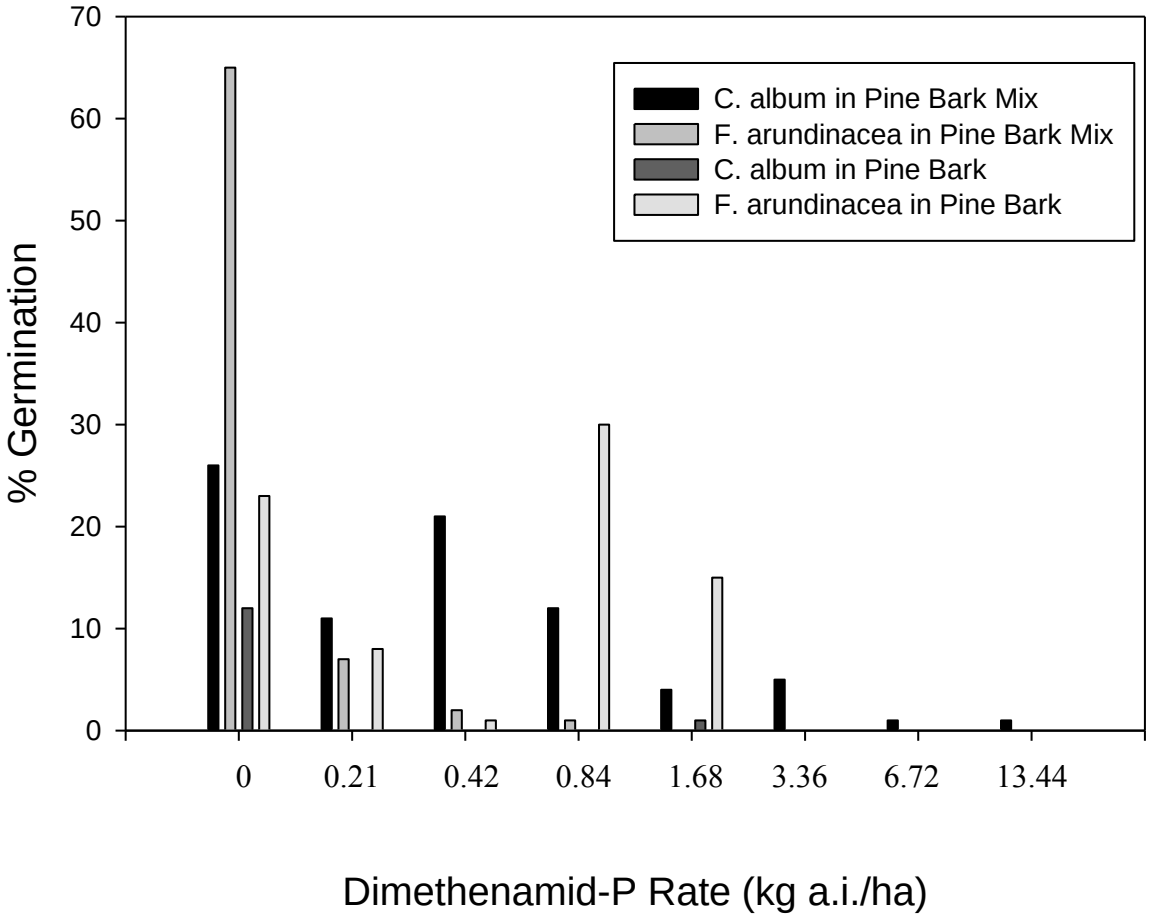


Figure 4.5. Percent germination of *C. album* and *F. arundinacea* grown in the soilless media treated with various rates of dimethenamid-P.



CHAPTER 5. SUMMARY AND CONCLUSIONS

Results from this study demonstrated that dimethenamid-P was detected by HPLC analysis in the leachate collected from the lysimeters with sand and no media, and the amount of dimethenamid-P recovered from these lysimeters were 46% and 61%, respectively. However, dimethenamid-P was not detected in pine bark and pine bark mix during any of the sample collections. Although this study indicated that there was no leaching in the media filled lysimeters, more research is needed to confirm these findings. With only one study, it is hard to be confident on making recommendations about the leaching potential of dimethenamid-P from the pine bark or pine bark mix. As with most preemergence herbicides, binding of the herbicide to the growing media accounts for the weed control of the preemergence herbicide.

It is likely that dimethenamid-P was bound to the two growing mediums chosen for the studies. Since the herbicide was able to control plant species for several weeks, this herbicide was available for seedling uptake and therefore the majority was still either adsorbed, absorbed, or simply stored within the media. In addition to adsorption, the fate of dimethenamid-P in the soilless media could be contributed to degradation and/or volatility. Microbial degradation, although unlikely as dimethenamid-P was never found in the leachate, and microbial breakdown would probably be a loss overtime. Another possibility is volatility, or the ability of an herbicide to change from a solid or liquid state to a gaseous state. Although, according to Senseman (2007), the tendency for dimethenamid-P to be lost due to volatilization is low. According to studies by Hargrove and Merkle (1971), alachlor, which is also a chloroacetamide herbicide, had decreasing losses to volatility with increases in relative humidity. In another study, around 50%

of alachlor and metolachlor volatilized from a glass surface following 8 days after application, while only 0.1% was lost from the soil surface (Parochetti, 1978).

The repeated bioassay demonstrated that dimethenamid-P provided adequate control of *Festuca arundinacea* for approximately 15 weeks in pine bark mix, while *Chenopodium album* control was more variable. The reason that dimethenamid-P provided inconsistent control of *C. album* can only be speculated. Again, dimethenamid-P was possibly bound to the growing media, degraded / broken down, volatilized, or a combination of all these process. Dimethenamid-P did however show significant control between the treated and untreated *Chenopodium album* and *F. arundinacea* grown in either media during the dose response study.

As with all research, there is always more that can be investigated and room for improvements in research and development. First of all, if time were permitted, a repeat of all studies would have been ideal. In future spray / lysimeters studies, all media should be nearly flush to the top of the lysimeters. Also critical, is knowledge, access, and maintenance of scientific equipment utilized in all studies. Time to test more weed species for germination and dimethenamid-P sensitivity could also have helped give a better idea of herbicide reactions using a range of weed species. Creating a watering system that simulated more of a normal rain event or nursery watering system could also be investigated to rule out the possibility of too much water in a short amount of time being applied to the system. In addition, looking into dimethenamid-P responses to soil versus soilless growing media could help us get a better idea of why the herbicide reacted the way it did in these growing substrates.

With all the uncertainties of the dimethenamid-P fate, it would be wise to look into the microbial breakdown process of dimethenamid-P in pine bark media and soil. What organisms are responsible and how long it takes to breakdown dimethenamid-P could be evaluated.

Measuring the major degradation products of dimethenamid-P (i.e. oxalamide) can also help determine the rate of microbial degradation. Vapor pressure could be measured using various gas chromatography methods to develop a better understanding of how much dimethenamid-P is lost due to volatility. For measuring adsorption, isotherms could be calculated using HPLC analysis and determining the amount of dimethenamid-P lost due to adsorption.

Although we did not find dimethenamid-P to leach in the pine bark and the pine bark mix in these studies, it does not mean that this compound could never leach. Applying dimethenamid-P to soilless growing media rather than soil caused the herbicide to react in a different manner. Attempting to apply the herbicide to just the surface of the container, and not the entire growing area, might help reduce the potential for leaching. Researchers must repeatedly put forth the effort to discover the fate of herbicides in a nursery situation, as soilless growing media and herbicide chemistries available for application will continue to expand and change.

SOURCE OF MATERIALS

[1]Fafard Inc., 770 Silver St., Agawam, MA 01001

[2] NSF International P.O. Box 130140 789 N. Dixboro Road Ann Arbor, MI 48113

[3] Boise Company, 3605 Warrensville Center Road, Shaker Heights, OH 44122

[4]Fisher Scientific, Pittsburgh, PA

[5]The Grieve Corporation, Round Lake, Illinois

[6]Industrial Wholesalers Incorporated, Griffin, GA 30223

[7]Oaty Supplies, Cleveland, OH

[8]GE Sealants and Adhesives, Huntsville, NC

[9]Phifer Incorporated, 4400 Kauloosa Avenue, Tuscaloosa, Alabama, 35401

[10]Netafim Ltd.**,** Derech Hashalom 10,Tel Aviv, Israel 67892

[11]Quikrete, One Securities Centre, 3490 Piedmont Road Suite 1300, Atlanta, GA 30305

[12]Brady® Labxpert™ Laboratory Labeling System

[13]Eppendorf Research Pippettes, Hamburg, Germany

[14]Argos Technologies1551 South Scottsdale Court, Suite 200, Elgin, IL 60123

[15]BASF Corporation, 100 Campus Drive, Florham Park, N.J. 07932

[16]R and D Sprayers, P.O. Box 267, Opelousas, LA 70571

[17]TeeJet Technologies 1801 Business Park Dr. Springfield, IL 62703-5626

[18]BD, Franklin Lakes, NJ

[19]Whatman, Springfield Mill, UK

[20]Chromatography Research Supplies Inc., Louisville, KY

[21]Büchi, Flawil, Switzerland

[22]Shimadzu, Kyoto, Japan

[23]ES Industries, West Berlin, NJ

[24]Azlin Seed, 112 Lilac Dr, Leland, MS 38756

REFERENCES

Abad, M., P. Noguera, S. Burés. 2001. National inventory of organic wastes for use as growing media for ornamental potted plant production: case study in Spain. Bioresource Technology. 77(2): 197-200.

Acquaah, G. 2005. Horticulture: Principles and Practices-Third Edition Upper Saddle River, New Jersey Prentice Hall.

Altland, J. 2003. Weed Control in Container Crops. Agrichemical Environmental News. 202.

Altland, J., M. Lanthier, USDA ARS. 2007. Influence of Container Mulches on Irrigation and Nutrient Management. Journal of Environmental Horticulture. 25: 234-238.

Anderson, K. A., J. L. Basile, E.R. Johnson. 2005. Analytical method for dimethenamid-P in selected raw agricultural commodities by gas chromatography with electron capture detection. Journal of AOAC International. 88(5): 1428-1432.

Atiyeh, R. M., C. A. Edwards, S. Subler, J.D. Metzger. 2000. Earthworm-Processed Organic Wastes as Components Of Horticultural Potting Media for Growing Marigold and Vegetable Seedlings. Compost Science & Utilization, JG Press, Inc. 8: 215.

Beardsell, D., D. Nichols, D.L. Jones. 1979. Physical properties of nursery potting-mixtures. Scientia Horticulturae. (Netherlands) 11: 1-8.

Beigel, C., M. P. Charnay, E. Barriuso. 1999. Degradation of formulated and unformulated triticonazole fungicide in soil: effect of application rate. Soil Biology and Biochemistry. 31(4): 525-534.

Bilderback, T. and M. Lorscheider. 1994. Physical properties of double-processed pine bark: Effects on rooting, ISHS. 77-84.

Billeaud, L. and J. Zajicek. 1989. Influence of mulches on weed control, soil pH, soil nitrogen content, and growth of Ligustrum japonicum. J. Environmental Horticulture. 7(4): 155-157.

Blythe, E. K. and D. J. Merhaut. 2007. Grouping and Comparison of Container Substrates Based on Physical Properties Using Exploratory Multivariate Statistical Methods. HortScience : a publication of the American Society for Horticultural Science. 42: 353-363.

Boatright, S. R. and J. C. McKissick. 2006. University of Georgia 2006 Farm Gate Value Report. AR-07-01.

Bobbitt, D. R. and S. W. Linder. 2001. Recent advances in chiral detection for high performance liquid chromatography. TrAC Trends in Analytical Chemistry. 20(3): 111-123.

Böger, P., B. Matthes, J. Schmalfuß. 2000. Towards the primary target of chloroacetamides -new findings pave the way. Pest Management Science. 56(6): 497-508.

Boger, P. 2003. Mode of action for chloroacetamides and functionally related compounds. Journal of Pesticide Science. 28(3): 324-329.

Bollman, S. L. and C. L. Sprague. 2007. Optimizing s-metolachlor and dimethenamid-P in sugarbeet microrate treatments. Weed Technology. 21(4): 1054-1063.

Bollman, S. L. and C. L. Sprague. 2008. Tolerance of 12 Sugarbeet Varieties to Applications of s-Metolachlor and Dimethenamid-P. Weed Technology. 22(4): 699-706.

Bollman, S. L., C. L. Sprague, D. Penner. 2008. Physiological basis for tolerance of sugarbeet varieties to s-metolachlor and dimethenamid-P. Weed Science. 56(1): 18-25.

Bryson, C. T., and M. S. Defelice, Ed. 2009. Weeds of the South. Athens, GA, University of Georgia Press.

Buamscha, M. G., J. E. Altland, D.M. Sullivan, D.A. Horneck. 2007. Micronutrient Availability in Fresh and Aged Douglas Fir Bark. HortScience. 42: 152-156.

Bunt, A. 1988. Media and mixes for container-grown plants: a manual on the preparation and use of growing media for pot plants, Unwin Hyman.

Buser, H.R. and M. D. Mueller. 1995. Environmental behavior of acetamide pesticide stereoisomers. 1. Stereo- and enantioselective determination using chiral high-resolution gas chromatography and chiral HPLC. Environmental Science & Technology. 29(8): 2023-2030.

Cedergreen, N. 2008. Herbicides can stimulate plant growth [electronic resource]. Weed Research 48: 429-438.

Couderchet, M., P. F. Bocion, R. Chollet, K. Seckinger, P. Boger. 1997. Biological Activity of Two Stereoisomers of the N-Thienyl Chloroacetamide Herbicide Dimethenamid. Pesticide Science. 50(3): 221-227.

Couderchet, M., J. Schmalfub, P. Boger. 1998. A specific and sensitive assay to quantify the herbicidal activity of chloroacetamides. Pesticide Science. 52(4): 381-387.

Crawford, J. J., G. K. Sims, F. W. Simmons, L. M. Wax, D. L. Freedman. 2002. Dissipation of the herbicide [C-14]dimethenamid under anaerobic conditions in flooded soil microcosms. Journal of Agricultural and Food Chemistry. 50(6): 1483-1491.

Czarnota, M. A. 2008. Personal communication. Dept. of Horticulture, University of Georgia, UGA Experiment Station, Griffin, GA, 30220-1797.

Deming, J. 1963. Determination of Volatility Losses of C^1 -CDAA from Soil Surfaces. Weed Science: 91-96.

Ebert, E. and K. Ramsteiner. 1984. Influence of metolachlor and the metolachlor protectant CGA 43089 on the biosynthesis of epicuticular waxes on the primary leaves of *Sorghum bicolor* Moench. Weed Research. 24(6): 383-389.

Eckermann, C., B. Matthes, M. Nimtz, V. Reiser, B. Lederer, P. Boger, J. Schroder. 2003. Covalent binding of chloroacetamide herbicides to the active site cysteine of plant type III polyketide synthases. Phytochemistry. 64(6): 1045-1054.

EPA. 2007. Chiral Chemistry: the ultimate in pollutant speciation. http://www.epa.gov/AthensR/research/process/chiralchemistry.html. Retrieved August 10, 2009.

Fain, G., P. Knight, C. H. Gilliam, J. W. 2003. Effect of Fertilizer Placement on Prostrate Spurge Growth in Container Production. Journal of Environmental Horticulture. 21(4): 177-180.

Fava, L., P. Bottoni, A. Crobe, E. Funari. 2000. Leaching properties of some degradation products of alachlor and metolachlor. Chemosphere. 41(9): 1503-1508.

Fehling, E. and K. Mukherjee 1991. Acyl-CoA elongase from a higher plant (Lunaria annua): metabolic intermediates of very-long-chain acyl-CoA products and substrate specificity. Biochimica et Biophysica Acta 1082(3): 239.

Fuerst, E. P. 1987. Understanding the Mode of Action of the Chloroacetamide and Thiocarbamate Herbicides.Weed Technology. 1(4): 270-277.

Gabriel, M. Z., J. E. Altland, J. S. Owen. 2009. The Effect of Physical and Hydraulic Properties of Peatmoss and Pumice on Douglas Fir Bark Based Soilless Substrates. HortScience. 44: 874-878.

Gallitano, L. and W. Skroch. 1993. Herbicide efficacy for production of container ornamentals. Weed Technology: 103-111.

Gotz, T. and P. Boger. 2004. The very-long-chain fatty acid synthase is inhibited by chloroacetamides. Zeitschrift fur Naturforschung. Section C, Biosciences. 59(7/8): 549-553.

Grey, T., G. Wehtje, B. F. Hajek, C. H. Gilliam, G. J. Keever, P. Pace. 1996. Adsorption, mobility, and filtration of metolachlor in container media. American Society for Horticultural Science. 121: 478-482.

Grey, T., M. Czarnota, T. Potter, B. T. Bunnell, USDA ARS. 2009. Timed Release of Flurprimidol from a Granular Formulation in Mulches and Sand. Hort Science. 44(2): 512-515.

Gronwald, J. W. 1991. Lipid Biosynthesis Inhibitors. Weed Science 39(3): 435-449.

Hamm, P. C. 1974. Discovery, Development, and Current Status of the Chloroacetamide Herbicides.Weed Science. 22(6): 541-545.

Handreck, K. and N. Black. 2002. Growing media for ornamental plants and turf, New South Wales Univ Pr Ltd.

Hargrove, R. S. and M. G. Merkle .1971. The Loss of Alachlor from Soil. Weed Science 19(6): 652-654.

Hartmann, H. T., D.E. Kester, F.T. Davies Jr., R.L. Geneve. 2002. Plant Propagation: Principles and Practices. Upper Saddle River, NJ Prentice Hall.

Heiskanen, J. 1999. Hydrological Properties of Container Media Based on Sphagnum Peat and their Potential Implications for Availability of Water to Seedlings after Outplanting. Scandinavian Journal of Forest Research, Taylor & Francis Ltd. 14: 78.

Horowitz, M. and C. L. Elmore. 1991. Leaching of Oxyfluorfen in Container Media. Weed Technology. 5(1): 175-180.

Hutchinson, P. J. S., C. V. Ransom, R. A. Boydston, B.R. Beutler. 2005. Dimethenamid-p: Efficacy and potato (*Solanum tuberosum*) variety tolerance. Weed Technology. 19(4): 966-971.

Ingram, D. L., R. W. Henley, T.H. Yeager. 1991. Environmental Horticulture Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. Growth Media for Container Grown Ornamental Plants. (BUL241).

Kalkhoff, S. J., D. W. Kolpin, E. M. Thurman, I. Ferrer, D. Barcello. 1998. Degradation of Chloroacetanilide Herbicides: The Prevalence of Sulfonic and Oxanilic Acid Metabolites in Iowa Groundwaters and Surface Waters. Environmental Science & Technology. 32(11): 1738-1740.

Kaufman, D. D. 1966. Microbial Degradation of Herbicide Combinations: Amitrole and Dalapon. Weeds 14(2): 130-134.

Keese, R. J., N. D. Camper, T. Whitwell, M. B. Riley, P. C. Wilson. 1994. Herbicide Runoff from Ornamental Container Nurseries. Journal of Environmental Quality. 23(2): 320-324.

Klein, C., R. J. Schneider, M. T. Meyer, D. S. Aga. 2006. Enantiomeric separation of metolachlor and its metabolites using LC-MS and CZE. Chemosphere. 62(10): 1591-1599.

Koren, E., C. L. Foy, F. M. Ashton. 1969. Adsorption, Volatility, and Migration of Thiocarbamate Herbicides in Soil. Weed Science 17(2): 148-153.

Koskinen, W. C., A. M. Cecchi, R. H. Dowdy, K. A. Norberg. 1999. Adsorption of Selected Pesticides on a Rigid PVC Lysimeter. Journal of Environmental Quality. 28(2): 732-734.

Laiche Jr, A. and V. Nash. 1986. Evaluation of Pine Bark, Pine Bark With Wood, and Pine Tree Chips as Components of a Container Plant Growing Media. Journal of Environmental Horticulture. 4(1): 22-25.

Le Baron, H. M., J.E. Mcfarland, B.J. Simoneaux, and E. Ebert. 1988. Herbicides: Chemistry, Degradation, and Mode of Action. e. P.C. Kerney and D.D. Kaufman. New York, Dekker. 3: 335-373.

Leonard, A. E., S. L. Pereira, H. Sprecher, Y. Huang. 2004. Elongation of long-chain fatty acids. Progress in Lipid Research. 43(1): 36-54.

Liu, L. C., H. Cibes-Viade, F. K. S. Foo. 1970. Adsorption of Ametryne and Diuron by Soils. Weed Science 18(4): 470-474.

Liu, W., J. Gan, D. Schlenk, W. A. Jury. 2005. Enantioselectivity in environmental safety of United States of America. 102(3): 701-706.

Ma, Y., W.P. Liu, Y.Z.Wen. 2006. Enantioselective Degradation of Rac-Metolachlor and S-Metolachlor in Soil. Pedosphere. 16: 489-494.

Mahnken, G., W. Skroch, T. J. Sheets, R. B. Leidy. 1994. Metolachlor and simazine leaching through horticultural substrates. Journal of Environmental Horticulture. 12: 55-58.

Maier, N. M., P. Franco, W. Linder. 2001. Separation of enantiomers: needs, challenges, perspectives. Journal of Chromatography. 906 (1-2): 3-33.

Matthes, B. and P. Böger. 2002. Chloroacetamides Affect the Plasma Membrane. Z. Naturforsch 57: 843-852.

McGregor, D., R. Solecki, T. E. Consultants, S. Aberdour. 2005. Dimethenamid-P/Racemic Dimethenamid. Joint Meeting on Pesticide Residues. Dimethandmid-P: 189-239.

Millar, A. and L. Kunst. 1997. Very-long-chain fatty acid biosynthesis is controlled through the expression and specificity of the condensing enzyme. Plant Journal. 12(1): 121.

Mueller, T. C., D. R. Shaw, W.W. Whit. 1999. Relative Dissipation of Acetochlor, Alachlor, Metolachlor, and SAN 582 from Three Surface Soils. Weed Technology. 13(2): 341-346.

Muller, M. D. and H.R. Buser. 1995. Environmental behavior of acetamide pesticide stereoisomers. 2. Stereo- and enantioselective. Environmental Science & Technology. 29(8): 2031.

Muller, M. D., T. Poiger, H. Buser. 2001. Isolation and Identification of the Metolachlor Stereoisomers Using High-Performance Liquid Chromatography, Polarimetric Measurements, and Enantioselective Gas Chromatography. Journal of Agricultural and Food Chemistry. 49(1): 42-49.

Muller, T. A. and H. P. E. Kohler. 2004. Chirality of pollutants - effects on metabolism and fate. Applied Microbiology and Biotechnology. 64(3): 300-316.

Neel, P. and F. Laudardale. 1972. Weed Control in Containers with Herbicide-Impregnated Mulch Materials. Proc. FL State Horticulture Society. 85(409-413).

Nelson, P. 1985. Greenhouse Operation and Management-Third Edition. Englewood Cliffs, NJ, Prentice-Hall. pp.3-26.

Osborne, B. T., D. R. Shaw, R. Ratliff. 1995. Response of Selected Soybean (Glycine max) Cultivars to Dimethenamid and Metolachlor in Hydroponic Conditions. Weed Technology. 9(1): 178-181.

Padgett, J. and T. Frazier. 1962. The relationship between costs and pruning of woody ornamentals. Ga. Agr. Expt. Sta. Bul. NS 100.

Parochetti, J. 1978. Photodecomposition, volatility, and leaching of atrazine, simazine, alachlor, and metolachlor from soil and plant material. WSSA Abstr. No. 17. Weed Science Society of America, Urbana, IL.

Peter, C. J. and J. B. Weber. 1985. Adsorption, Mobility, and Efficacy of Alachlor and Metolachlor as Influenced by Soil Properties. Weed Science 33(6): 874-881.

Prueger, J. H. and R. L. Pfeiffer. 1994. Preliminary Tests of a Laboratory Chamber Technique Intended to Simulate Pesticide Volatility in the Field. Journal of Environmental Quality 23(5): 1089-1093.

Retzinger, E. J., Jr. and C. Mallory-Smith. 1997. Classification of Herbicides by Site of Action for Weed Resistance Management Strategies. Weed Technology. 11(2): 384-393.

Riechers, D. E., E. P. Fuerst, K. D. Miller. 1996. Initial Metabolism of Dimethenamid in Safened and Unsafened Wheat Shoots. Journal of Agricultural and Food Chemistry. 44(6): 1558-1564.

Riley, M. B., R. J. Keese, N. D. Camper, T. Whitwell, P. C. Wilson. 1994. Pendimethalin and Oxyfluorfen Residues in Pond Water and Sediment from Container Plant Nurseries. Weed Technology. 8(2): 299-303.

Robinson, D. E., K. McNaughton, N. Soltani. 2008. Weed Management in Transplanted Bell Pepper (Capsicum annuum) with Pretransplant Tank Mixes of Sulfentrazone, S-metolachlor, and Dimethenamid-p. HortScience. 43: 1492-1494.

Saito, K., M. Yato, T. Ito, Y. Iwasaki, R. Ito, Y. Matsuki, H. Nakazawa. 2008. Verification of the need for optical purity measurement of chiral pesticide standards as agricultural reference

materials. Accreditation and Quality Assurance: Journal for Quality, Comparability and Reliability in Chemical Measurement. 13(7): 373-379.

Sakaliene, O., S. K. Papiernik, W. C. Koskinen, I. Kavoliul, J. Brazenaieti. 2009. Using Lysimeters To Evaluate the Relative Mobility and Plant Uptake of Four Herbicides in a Rye Production System. Journal of Agricultural and Food Chemistry. 57(5): 1975-1981.

Schmalfub, J., B. Matthes, P. Mayer, P. Boger. 1998. Chloroacetamide mode of action, I: Inhibition of very long chain fatty acid synthesis in Scenedesmus acutus. Zeitschrift Fur Naturforschung C. 53: 995-1003.

Schmalfub, J., B. Matthes, K. Knuth, P. Boger. 2000. Inhibition of Acyl-CoA Elongation by Chloroacetamide Herbicides in Microsomes from Leek Seedlings. Pesticide Biochemistry and Physiology. 67(1): 25-35.

Seefeldt, S., J. Jensen, E. P. Fuerst. 1995. Log-logistic analysis of herbicide dose-response relationships. Weed Technology: 9: 218-227.

Senseman, S. A., Ed. 2007. Herbicide Handbook, 9th ed. Weed Science Society of America. Lawrence, Kansas. pp. 14, 262-262, 420.

Simmons, L. D. and J. F. Derr. 2007. Pendimethalin Movement Through Pine Bark Compared to Field Soil. Weed Technology. 21(4): 873-876.

Sorokina, M. N. and G. W. Thomas. 1997. Imazaquin Leaching in Karnak Soil in Kentucky. Weed Science 45(5): 722-726.

Stamps, R. H. 1997. Herbicides labeled for greenhouse use. GPN: Greenhouse Product News 7(6): 8.

Szmedra, P. 1997. Banning 2,4-D and the Phenoxy Herbicides: Potential Economic Impact. Weed Science 45(4): 592-598.

Tadeo, J. L., C. Sánchez-Brunete, R. A. Perez, M. D. Fernandez. 2000. Analysis of herbicide residues in cereals, fruits and vegetables. Journal of Chromatography. 882(1-2): 175-191.

Thurman, E. M., D. A. Goolsby, M. Meyer, M. Mills, M Pomes, D. W. Kolpin. 1992. A reconnaissance study of herbicides and their metabolites in surface water of the midwestern United States using immunoassay and gas chromatography/mass spectrometry. Environmental Science & Technology. 26(12): 2440-2447.

University of Kentucky-College of Agriculture, C. E. Issued 2004. Container Nursery Production. http://www.uky.edu/Ag/NewCrops/introsheets/container.pdf. Retrieved November 4, 2008.

USDA. 2007. Census Publications: Volume 1, Chapter 2, Table 35. Nursery, Greenhouse, Floriculture, Sod, Mushrooms, Vegetable Seeds, and Propagative Materials Grown for Sale: 2007 and 2002. Retrieved August 10, 2009.

Uva, R. H., J. C. Neal, J. M. DiTomaso. 1997. Weeds of the Northeast. Cornell University Press. Ithaca, NY. pp: 96, 204, 206.

Vasilakoglou, I. B., I. G. Eleftherohorinos, K. B. Dhima. 2001. Activity, adsorption and mobility of three acetanilide and two new amide herbicides. Weed Research, Blackwell Publishing Limited. 41: 535-546.

von Wettstein-Knowles, P. 1982. Elongase and epicuticular wax biosynthesis. Physiology of Vegetables. 20: 797-809.

Wallace, R. W. and J. C. Hodges. 2007. Weed Control Potential and Crop Safety of Selected Herbicides in Field-grown Cannas. HortTechnology. 17: 102-106.

Weber, J. B. 1990. Behavior of Dinitroaniline Herbicides in Soils. Weed Technology 4(2): 394-406.

Wehtje, G., J. E. Altland, C. H. Gilliam, S. C. Marble, A. van Hoogmoed, G. B. Fain. 2009. Weed Growth and Efficacy of PRE-Applied Herbicides in Alternative Rooting Substrates Used in Container-Grown Nursery Crops. Weed Technology. 23(3): 455-459.

Williams, A. 1996. Opportunities for chiral agrochemicals. Pesticide Science. 46(1): 3-9.

Winton, K. and J. B. Weber. 1996. A Review of Field Lysimeter Studies to Describe the Environmental Fate of Pesticides. Weed Technology 10(1): 202-209.

Wong, C. 2006. Environmental fate processes and biochemical transformations of chiral emerging organic pollutants. Analytical and Bioanalytical Chemistry. 386(3): 544-558.

Yokley, R. A., L. C. Mayer, S. Huang, J. D. Vargo. 2002. Analytical Method for the Determination of Metolachlor, Acetochlor, Alachlor, Dimethenamid, and Their Corresponding Ethanesulfonic and Exanillic Acid Degradates in Water Using SPE and LC/ESI-MS/MS. Analytical Chemistry. 74(15): 3754.

Zimdahl, R. L. and S. K. Clark. 1982. Degradation of Three Acetanilide Herbicides in Soil. Weed Science. 30(5): 545-548.

Zimmerman, L., R. Schneider, E. M. Thurman. 2002. Analysis and detection of the herbicides dimethenamid and flufenacet and their sulfonic and oxanilic acid degradates in natural water. Journal of Agricultural and Food Chemistry. 50(5): 1045-1052.