

ABSTRACT

Title of Thesis: THATCH AND SOIL PESTICIDE
 DEGRADATION AND MICROBIAL
 ACTIVITY AS INFLUENCED BY
 TURF CULTIVATION PRACTICES

Yusong Mu, Master of Science, 2009

Thesis Directed By: Mark J. Carroll, Ph.D.
 Associate Professor
 Department of Plant Science and Landscape
 Architecture

Pesticide degradation in turf is complicated by presence of an organic matter enriched layer called thatch. It is not well understood how the extensive pesticide sorption capacity of thatch may affect the aerobic degradation of pesticides in thatch. Hollow tine cultivation and vertical mowing are two commonly used cultivation practices used to control thatch. Two studies were conducted to determine how these two cultural practices may affect microbial activity and pesticide degradation within thatch and soil. Hollow tine cultivation briefly enhanced microbial activity within thatch while vertical mowing had no consistent effect on thatch or soil microbial activity. Neither cultivation practice consistently altered the aerobic degradation of 2,4-D, flutolanil or chlorpyrifos. Thatch and soil aerobic degradation constants obtained for flutolanil and chlorpyrifos supported the hypothesis that strongly

adsorbed pesticides are shielded from microbial populations that degrade pesticides within thatch.

THATCH AND SOIL PESTICIDE DEGRADATION AND MICROBIAL
ACTIVITY AS INFLUENCED BY
TURF CULTIVATION PRACTICES

By

Yusong Mu

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Advisory Committee:
Dr. Mark J. Carroll, Advisor
Dr. Peter Dernoeden
Dr. Thomas Turner

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CHAPTER I. INTRODUCTION

Considerable acreages of turfgrasses are maintained in the landscape at various intensities. On highly managed turf sites such as golf courses and athletic fields, intensive cultural practices are performed on a regular basis to maintain desirable playing surface characteristics. Such practices may involve frequent mowing, turf cultivation, and the reduction of turfgrass pests through the use of pesticides.

Turfgrass is a unique plant community compared with other agronomic or landscape plant communities in that a tightly intermingled layer of dead and living plant material called thatch can develop between turfgrass foliage and the soil surface. Excessive thatch buildup is frequently associated with the occurrence of localized dry spots and an increase in some disease and insect problems. Excess thatch can also reduce pesticide effectiveness and result in a general decline in turf quality.

Hollow tine cultivation and vertical mowing are two mechanical cultivation practices that are commonly used to reduce thatch accumulation. Thatch is removed when soil cores are pulled up by the hollow tines of a cultivator, or alternatively when the vertically rotating blades of a vertical mower slice into thatch and bring plant debris up from below the turf canopy. It is often stated that hollow tine cultivation and vertical mowing can bring about improved biological conditions within thatch. One such claim is that the addition or incorporation of soil into thatch will enhance microbial activity within thatch. The introduction of soil can occur when cores removed by hollow tine cultivation are incorporated into the turf rather than being

removed from the site where the cores were extracted. Past research involving hollow tine cultivation or vertical mowing have largely focused on how these two practices affect thatch accumulation. No published research appears to have directly examined how these two cultivation practices may alter microbial activity within thatch. Knowledge of the effect of hollow tine cultivation and vertical mowing on thatch microbial activity will help turf managers better understand the roles these two cultivation practices have in managing turf.

In order to maintain the functional ability of a turf surface, pesticides are frequently used to eliminate weed encroachment and to prevent the occurrence of diseases and insect pests that can be injurious to turf growth. Before a pesticide can be approved for use on turf or any other crop, a thorough examination of the ecological hazards associated with the use of the pesticide must be conducted by the registrant. This is done in conjunction with the regulatory agency that ultimately grants approval for use of the pesticide. One essential piece of information that must be submitted as part of the pesticide registration package is the aerobic half life of the pesticide. The aerobic half life of a pesticide is a key indicator of the potential persistence of the pesticide in the environment. As such it frequently serves as the persistence parameter in models that are used to assess the environmental fate of a pesticide.

Microbial degradation is often the primary process that regulates the aerobic decay of a pesticide. Thatch, because of its higher organic matter content, supports higher microbial populations than most mineral soils. The high microbial populations found within thatch have been used as an explanation to account for faster rates of

pesticide dissipation seen in turf than in agricultural soils. Pesticide aerobic degradation is typically measured in batch incubation experiments. Few batch incubation experiments have investigated the degradation of pesticides in thatch. The few thatch pesticide incubation studies that have been published suggest that enhanced pesticide degradation in thatch is limited to pesticides having low to moderate sorption affinities. This is consistent with numerous mineral soil based studies that have determined sorption shields pesticides from microbial degradation. The results of past thatch incubation investigations also appear to indicate that the presence high microbial populations in thatch may be of little consequence in the degradation of pesticides that are almost entirely sorbed to the organic matter present in thatch.

Thatch is the primary porous media sorbent of pesticides that are applied to turf. Given the important role thatch has in retaining pesticides, a better understanding of the interaction that exists between pesticide sorption and pesticide degradation within thatch is needed. This is especially true in light of the fact that only a single incubation study appears to exist in the literature that has examined the degradation of pesticides having contrasting sorption properties (Frederick et al., 1994). A more complete understanding of pesticide microbial degradation in thatch would aid modelers in deciding how to adjust the United State Department of Agriculture, Agriculture Research Service (USDA-ARS) pesticide dissipation degradation constants to better represent how thatch influences the persistence of pesticides that are applied to turf. The USDA-ARS pesticide dissipation data base was developed by relying almost exclusively on investigations involving mineral soils.

Hollow tine cultivation and vertical mowing may alter microbial activity in thatch and soil. If microbial activity is influenced by these two cultivation practices, microbial degradation of turf-applied pesticides may also be affected. Two studies were conducted to investigate how turf cultivation may influence thatch and soil microbial activity and pesticide degradation. The first study was a field based study conducted at two locations where the microbial activity in thatch and soil was examined over a 56 to 58 day period following the imposition of three cultivation treatments. The primary objective of this study was to determine if hollow tine cultivation and vertical mowing altered thatch and soil microbial activity. The second study was a pesticide laboratory incubation study that examined the effect hollow tine cultivation and vertical mowing on the degradation of three pesticides having differing sorption affinities in thatch and soil. The primary objective of the second study was to determine if the sorption properties of a pesticide altered the relative persistence of the pesticide in thatch and soil.

CHAPTER II. LITERATURE REVIEW

2.1. PESTICIDE USE IN TURF MANAGEMENT

Turfgrass is the predominant feature found in urban and suburban landscapes. Recent surveys of the turf industry within the highly urbanized States of Maryland and New Jersey for example have reported 18 percent of the total land area in these states exists as turfgrass (2005 Maryland Turfgrass Survey, 2007 New Jersey Turfgrass Survey). It has been estimated that the average lawn size for a homeowner in the United States is approximately 1,200 square meters (Vinlove and Torla, 1995) while the average golf course in the U.S. has 40 hectares of turf that is managed at varying levels of intensity (Lyman et al., 2007).

Turfgrass pests often create problems that can lead to dramatic declines in turf quality. Loss in turf quality can impair some functional attributes associated with turf use in the landscape. Declines in turf density arising from disease and insect damage can diminish the runoff and chemical filtration properties of a previously dense turfgrass sward. Easton and Petrovic (2004) found that a 50% reduction in shoot density (i.e., from 10 to 5 shoots cm^{-2}) resulted in three fold increase in runoff. These same authors also noted that a turf's ability to reduce nutrient runoff losses increases with increasing shoot density.

Reseeding a sward is one option that can be used to restore turf density. However, it is often more cost effective to rely on pesticides to minimize turf damage from pests that are a recurring problem. When applied on a preventive schedule, insecticides can prevent severe thinning of turf that would otherwise occur at a site

that has a history of repeated white grub infestations (Watschke et al., 1994). Pesticide use also is frequently required for aesthetics reasons. Visual decline in turf resulting from pest infestation can affect the playability of a sports turf surface and the overall beauty of an otherwise problem-free home lawn turf. Herbicides, fungicides, and insecticides, when used selectively are beneficial tools that will help ensure turf density and health. This can be particularly important for golf courses where pesticide use is warranted to maintain the functional performance of fine golf turf.

Pesticides can be indispensable tools for various pest management circumstances, especially when no alternative is available. This is especially true when the pest problem is so severe that a “pesticidal rescue” application is needed. Many pesticides provide very effective pest control, and their use has evolved in some situations to the point where they are the first line of defense in a turf pest control program (Watschke et al., 1994).

Integrated Pest Management (IPM) is a management approach that includes, but does not limit, pest control measures to the use of pesticides. Integrated Pest Management relies on a combination of cultural, biological, and chemical practices to control pests. One of the primary goals in the development of Integrated Pest Management practices is to reduce overall pesticide use. In a recent study that evaluated reduced chemical management systems for putting green turf, it was found that IPM greens received 27-46% fewer pesticide applications than unrestricted pest management greens (Grant and Rossi, 2004). Examples of IPM practices in this particular study included rolling greens in the morning to reduce incidence and

severity of dollar spot (caused by *Sclerotinia homoeocarpa* F. T. Bennett), manual removal of weeds, and tree removal to increase sunlight and air circulation on the greens. Reduced reliance on use of pesticides to maintain turf quality is frequently accompanied by using disease-resistant cultivars, maintaining high mowing heights, irrigating deeply and infrequently, and using a balanced fertility program (Leslie, 1994).

Potential environmental and public health risks associated with the use of pesticides in turf have been a concern of the general public for several decades. For example, lawns treated with pesticides have been described as “toxic lawns” and it is commonplace for the general public to have negative impressions on the use of pesticides on home lawns and golf courses (Gruen, 2007). Public and regulatory concerns have focused not only on the potential misapplication of pesticides but the risks associated with routine use of pesticides in turfgrass as well (Cisar and Snyder, 1996).

Much of the concern associated with turf pesticide use centers on its potential risks to human health. One of the major health effects of concern is the relationship between pesticide use and the occurrence of cancer. Recent studies seemed to suggest a possible association between the incidence of lung cancer and the commonly used turf pesticide chlorpyrifos [O,O-diethyl-O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate] (Alavanja et al., 2004a; Lee et al., 2004), and a potential link between methyl bromide and increased risk of prostate cancer (Alavanja et al., 2003). A recent review examined the carcinogenicity and genotoxicity of pesticides commonly used on golf courses (Knopper and Lean, 2004). The authors suggested a

possible relationship between the use of certain pesticides on golf courses, such as iprodione [3-(3,5-dichlorophenyl)-N-(1-methylethyl)-2,4-dioxo-1-imidazolidine-carboxamide], chlorothalonil (tetrachloroisophthalonitrile), and 2,4-D (2,4-dichlorophenoxyacetic acid), and cancer in humans and wildlife. The review also suggested an increasing potential for unintentional human and animal exposure to turf pesticides with new golf courses being created each year. Human exposure can occur from direct inhalation of the active ingredient of the pesticide, through direct contact with treated plant surfaces, or through drinking the water that may have been contaminated by pesticides. Pesticide exposure has been linked to neurological problems, lung damage and birth defects (Alavanja et al., 2004b).

To reduce the risks associated with pesticide use, chemical companies have developed and brought to market plant protection chemicals that require much lower use rates than historically once used. The load of active ingredient of many herbicides applied to turf nowadays has been reduced 50 to 100 times, compared with application rates 10 to 15 years ago (Branham et al., 1995). For example, the current application rate of chlorsulfuron {2-chloro-*N*-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl]-benzenesulfonamide}, a selective herbicide that controls grassy and broadleaf weeds in turf, is as low as 113 grams a.i./acre (Nufarm Turf & Specialty, 2005). On the other hand, the application rate of siduron [1-(2-methylcyclohexyl)-3-phenylurea], a herbicide for control of certain annual weed grasses in turf is 3630-5440 grams a.i./acre (PBI Gordon Corporation, 2002).

New agrochemicals also target pests with increased specificity, reducing potential risks to non-target organisms, humans, and the environment. Recent

advances in structure-based design of agrochemicals have allowed tests of a large numbers of compounds (> 100,000 per year) for whole organism activity against a variety of target species including plants, fungi and insects (Walter, 2002). This has facilitated the discovery of molecules having defined biological activity against a specific target species. For example, the insecticide imidacloprid [1-(1-[6-chloro-3-pyridinyl]methyl)-N-nitro-2-imidazolidinimine] causes insecticidal toxicity by affecting the insect nervous system, resulting in excitation and uncoordinated movements of the target insect but has relatively low mammalian toxicity (Buckingham et al., 1997). Additionally, the development of short-persistence pesticides is allowing turf pest problems to be sufficiently controlled at lowered costs to the environment. There are risks overall, but the tangible agronomic benefits of turf pesticides make them useful tools in maintaining turfgrasses.

2.2. ENVIRONMENTAL FATE OF PESTICIDES

2.2.1. Pesticide dissipation

The behavior of a pesticide results from the interactions between the chemical and various components of the environment. Pesticides can undergo various routes of dissipation once applied in the environment. Major routes of pesticide dissipation include adsorption, transfer (i.e. volatilization, runoff, leaching, absorption) and degradation (i.e. photodegradation, chemical degradation, and microbial degradation).

Adsorption occurs when the pesticide binds to soil particles. Soil sorption is the affinity a chemical has to adhere to soils. The extent to which a pesticide is adsorbed to soil depends on soil type, soil texture, soil pH, soil moisture and the pesticide itself

(Parker and Doxtader, 1983; Wild, 1993; Gan et al., 1996; Dyson et al., 2002). Soil retention characteristics of pesticides, for example, vary with the number and location of polar functional groups of the pesticides. Cationic pesticides are strongly held to negatively charged soil by ionic bonds. Anionic pesticides are poorly held to negatively charged soil particles unless positively charged soil colloids are present; and nonionic pesticides are often weakly held at the soil surfaces through weak physical forces (McCarty et al., 2003). Clay or organic soils are more adsorptive than coarse, sandy soil due in part to their increased surface areas (Johnson et al., 2007). Soil pH can affect the equilibrium between undissociated pesticide molecules and the anion molecules of the pesticide. Such an equilibrium shifts as soil pH changes in relation to pK_a value of the pesticide. The herbicide 2,4-dichlorophenoxyacetic acid, for example, has a pK_a of 2.8 (Wauchope et al., 1992). When soil pH goes above 2.8, 2,4-dichlorophenoxyacetic acid would exist primarily in its dissociated, negatively charged form. As soil pH increases, adsorption will decrease because the 2,4-D molecules are more repelled from the overall negative charges of soil colloids (McCarty et al., 2003).

Pesticide volatilization occurs when the solid or liquid form of a pesticide is transformed into a gas. Volatilization can reduce effectiveness of a pesticide by reducing the amount of chemical that makes contact with the intended target. As temperature and ambient air movement increase, the potential for pesticide loss through volatilization increases (Yates et al., 2002; Haith et al., 2002). Henry's law summarizes the relationship between pesticide solubility and volatilization by stating that pesticide volatile loss is inversely proportional to pesticide solubility (Dearden

and Gerrit, 2003). Pesticide loss in the air can also occur because of spray drift. Drift consists of droplets produced by nozzles of the spray equipment being suspended in air and carried away by air flows before reaching any surface.

Pesticide runoff occurs when pesticides are carried away by surface water movement. Typically, if water addition to a field is faster than it can be absorbed into the soil, runoff occurs. Pesticide molecules can move either when dissolved in the water, or through attachment with soil particles or sediments (Bailey et al., 1974). Factors that influence pesticide runoff include the physic-chemical properties of the pesticide, application method, soil property, hillside slope, timing, duration, intensity, climate, and agricultural practices (Zhang et al., 1997; Lecomte et al., 2001; Chaplot et al., 2003). Losses from runoff can be greatest when it rains heavily immediately after a pesticide application (Smith and Bridges, 1996; Ma et al., 1999). For example, Ma et al. (1999) simulated rainfall 1 d before and 1,2,4, and 8 d after field application of 2,4-D [(2,4-dichlorophenoxy) acetic acid], dicamba (3,6-dichloro-2-methylphenoxy-benzoic acid), and mecoprop [(±)-2-(4-chloro-2-methylphenoxy)-propanoic acid]. They found that both the mass and concentration of pesticide runoff decreased rapidly with each rainfall event, with the first posttreatment event runoff averaging 74.5, 71.7, and 73.0% of the total runoff of 2,4-D, dicamba, and mecoprop, respectively.

Leaching is the downward movement of chemicals in water through the soil. Pesticides that are easily leached have a high potential to reach groundwater. The characteristics of the soil and pesticide play an important role in influencing pesticide leaching. Sandy soils are more prone to leaching than clay textured soils because

macropore flow in sandy soil is more extensive than in clay textured soil. When macropore flow is less extensive, adsorption is stronger and leaching potential is lower (Roulier and Jarvis, 2003). Pesticides having low water solubility, high soil adsorption, and low persistence are less likely to leach than pesticides that are highly soluble, less adsorptive to soil, and more persistent (Webb et al., 2008; Magri and Haith, 2009).

Absorption is the movement of chemicals from the surface to the interior of the plant. Plant absorption can occur either through leaves or roots of the plant. Systemic pesticides, for example, move inside a plant following absorption of the plant.

Degradation is the process of pesticide breakdown after application and it is a very important route of pesticide dissipation. As pesticides are broken down, the possibility of the pesticide chemicals reaching ground or surface water and thus creating environmental or health related concerns is generally minimized. Pesticides can be broken down by microbes, chemical reactions, and light; these processes are known as biodegradation, chemical degradation, and photodegradation, respectively (Wheeler, 2002).

Microbial degradation is the breakdown of pesticides by fungi, bacteria, and other microorganisms that use pesticides as an energy source. The aerobic and anaerobic oxidation and reduction of pesticides by microbial populations produce energy for the microbes (Magri and Haith, 2009). In soil-enriched environments, soil conditions such as moisture, temperature, pH, and the amount of organic matter affect the rate of microbial degradation due to their direct or indirect influence on microbial growth and microbial activity (Wise and Trantolo, 1994; Magdoff and Weil, 2004).

Chemical degradation is the breakdown of pesticides by processes where living organisms are not involved. Major chemical reactions such as hydrolysis, oxidation, and reduction, without the influence of microbial activity, are processes involved in chemical degradation.

Photodegradation is the breakdown of pesticide by sunlight. The rate of breakdown is influenced by the intensity and spectrum of sunlight, length of exposure, and the properties of the chemical. Photodegradation can occur by direct or indirect absorption of light (Zepp and Cline, 1977). In direct photolysis, for example, the substance absorbs UV-visible light energy and undergoes transformation (Konstantinou et al., 2001).

2.2.2. Pesticide microbial decay

i) Methods used to evaluate microbial decay

Pesticide microbial degradation is a key process attenuating pesticide fate in the environment. Pesticides are broken down by microorganisms in the soil through a series of reactions, leading to the eventual production of carbon dioxide, water, and possibly some inorganic products. In the early stages of microbial degradation the parent compound is transformed into one or more new compounds that may have different chemical and physical properties.

Various methods have been developed to evaluate pesticide microbial decay. Microecosystems, also known as microcosms, have been used to study the fate of pesticide applied to plant stands. Metcalf et al. (1971) developed a microecosystem that followed the movement of pesticides and metabolites through a food chain. This

food chain, with *Sorghum* plants as its primary producer, had six other elements and a terrestrial-aquatic interface, enabling the simulation of pesticide applications to crop plants and the eventual contamination of the aquatic environment. Radiolabeled pesticides were applied to the system and evaluated via components of the food chain. The Metcalf et al. (1971) microecosystem allowed for the examination of the ecological fate of pesticides. It was particularly useful in studying the metabolic transformations of pesticides in the various elements of the system. Food chain-based model ecosystems require careful selection of a series of organisms that are compatible with the conditions in the terrestrial-aquatic system and may not be an appropriate method when pesticide microbial degradation is the primary research interest. The food chain-based model ecosystem approach does not offer a straight forward interpretation of pesticide microbial degradation because of the various food chain components making up the ecosystem.

Microecosystems emphasizing the examination of specific pesticide dissipation pathways have been developed. For example, a closed microecosystem was designed for the purpose of studying pesticide fate in turfgrass by Branham et al. (1985). Their system consisted of a media-base located in an enclosed atmospheric chamber and an analytical trapping system. The media-base supported plant growth, simulated field drainage conditions, and collected leachate of applied chemicals. The trapping system they created captured volatilized pesticide and volatilized CO₂ resulting from pesticide microbial degradation. Using this microecosystem, Branham et al. (1985) found that three weeks after the application of radiolabeled diazinon [0,0-diethyl-0-(2-isopropyl-6-methyl-4-primidiny) phosphorothioate], 47% of the ¹⁴C label

remained in the form of the parent compound, 22% had been metabolized and lost as $^{14}\text{CO}_2$, 1% had leached through the profile, 2% had been lost through volatilization, and 28% remained in the soil as a metabolite or as one or more unextractable compounds.

The analytical trapping system of a microecosystem such as that in Branham et al. (1985) can be altered to accommodate the need of a particular compound and the dimensions of microecosystem altered to fit certain research needs. The total accountability of a chemical and its metabolites is possible when a well designed microecosystem is used to monitor ^{14}C leaching and volatile losses. One disadvantage with this kind of system is that no measurement of photodegradation is possible if the atmospheric chamber is made of glass as it is opaque to ultraviolet light.

Researchers have used soil respiration from incubated soils as a means to indicate overall microbial activity for the purpose of determining pesticide degradation. Guo et al. (1999), for example, measured total CO_2 evolved from an incubated soil. The CO_2 evolved from the soil was trapped by a KOH solution and the amount of CO_2 determined by titration. This method assumed that the absorption efficiency of CO_2 by KOH was 100% and the amount of CO_2 evolved was associated with soil microbial activity, and thus an indication of the extent to which pesticide degradation occurred. A pesticide can also be radioactively labeled for the purpose of generating labeled $^{14}\text{CO}_2$. For example, Langner et al. (1998) examined [^{14}C]2,4-D degradation by determining the fraction of total added ^{14}C recovered as $^{14}\text{CO}_2$. Such CO_2 trapping methods facilitate measurements of the overall microbial activity in an incubated soil without taking separate soil samples. The amount of CO_2 evolved is the

only parameter measured in this system. The use of this approach does not identify which metabolites are formed when degradation of the parent compound occurs. Thus the results obtained cannot be used to generate an accurate description of transformations that occur in the applied compound's degradation process.

Pesticide microbial decay can be examined via field based degradation studies. This is carried out by making on-site pesticide applications and quantifying collected field samples for chemical residues. However there are several disadvantages associated with conducting field based degradation studies. First, there is field-scale spatial variation in organic carbon content. Such variations have a major influence on the variability of the microbial parameters, sorption, and pesticide mineralization in the soil (Vinther et al., 2008). Second, there are uncontrollable variations in weather conditions. Soil moisture and temperature conditions likely will keep fluctuating during the duration of a field based study. Degradation of a pesticide under inconstant soil conditions can be different from degradation of the same pesticide under controlled soil conditions because of the possible difference in the behavior of pesticide degrading microbes in these two scenarios. Third, it is not practical to isolate microbial losses of the pesticide from other avenues of losses under typical field conditions. Such difficulties are inherent with field based degradation studies forcing researches to rely on laboratory incubation investigations to examine pesticide microbial decay under constant conditions.

Laboratory based incubation studies provide the opportunity to investigate pesticide microbial decay under constant environmental conditions. Environmental parameters such as soil texture, moisture, temperature can be easily controlled in

laboratory incubation investigations. In addition, by comparing sterilized sample pesticide losses with non-sterilized sample pesticide losses, laboratory based incubation studies can be used to separate microbial decay from other avenues of pesticide losses (Getzin and Rosefield, 1968; Racke et al., 1996; Roy et al., 2001).

Typically, the soil material used in a pesticide incubation study consists of homogenized disturbed soil material or intact soil cores. The use of disturbed soil material involves placing the material into a vessel after which one or more pesticides are added to the vessels. Alternatively, when intact soil cores are used, pesticides are added to the upper surface of the cores and allowed to disperse downward within the cores. Incubation is carried out under constant moisture and temperature conditions by periodically adding appropriate amounts of water to the incubated sample materials that are kept inside a controlled temperature incubator. A measurement of pesticide degradation can be obtained by extracting the pesticide chemicals at desired time intervals and running the extractants through gas or liquid chromatography to determine residue concentrations. Incubations using disturbed and homogenized soil materials are commonly referred to as batch incubation. Compared with the heterogeneous, intact soil cores through which soil water moves, the soil in batch systems is hydrodynamically static. Although both approaches have been used to study pesticide degradation, the use of intact soils may give results that are more consistent with field data because intact soil cores are a better representation of field conditions (Gaston et al., 2003).

Pesticide degradation data are often obtained from simple laboratory experiments with the assumption that lab-based results can be extrapolated to field

conditions. Assumptions inherent in using laboratory generated decay constants to represent pesticide decay in the field include: 1) laboratory study results obtained with disturbed soil and constant temperature and moisture regimes are characteristic of pesticide field degradation in structured soil with fluctuating moisture and temperature; and 2) degradation in the static soil systems in the laboratory is identical to degradation under flow conditions in the field (Beulke et al., 2005). Such assumptions may not be valid in cases where laboratory processing of field sample materials may have dramatically changed soil properties, in which case microbial access to the pesticide may be substantially different than what would occur under field conditions. Additionally, the use of individual or a small number of soil samples in most cases will not adequately assess the variable nature of pesticide decay in the field that occurs as a result of spatial changes in chemical and physical properties of the medium in which the pesticide has been applied (Beulke et al., 2005).

ii) Factors that affect microbial activity

Pesticide biodegradation is inanimately tied to the activity of specific microorganisms. Soil temperature and moisture are important factors that influence the activity of soil microorganisms. Numerous studies have examined the effects of soil temperature and moisture on the biodegradation of agrochemicals in soils (Guenzi and Beard, 1975; Parker and Doxtader, 1983; Choi et al., 1988; Veeh et al., 1996). Generally, faster pesticide degradation rates occur with increasing soil temperature up to a temperature that corresponds to the maximal activity of the microorganisms that use the pesticide as a substrate (Choi et al., 1988; Ma et al.,

2001). Once temperature goes beyond an optimum level, degradation rates decline (Parker and Doxtader, 1983; Veeh et al., 1996). For example, in a laboratory experiment involving six soil temperatures, the rate of DCPA (dimethyl tetrachloroterephthalate) degradation was influenced by soil temperature in the following order: $10 \ll 15 \ll 20 < 25 = 30 > 35$ °C; with the average half life ranging from 92 d at 10 °C to 18 d at 30 °C (Choi et al., 1988). The temperature range associated with maximum pesticide degradation corresponded to the soil temperature range (i.e., 25 – 30 °C) commonly associated with optimal growth for a wide range of soil microorganisms (Elsas et al., 2007). Similar results have been obtained with other xenobiotics as well. In a study investigating the degradation of DDT [(1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane)] in flooded soil, 80, 64, 44, 10 and 48% of the amount applied was recovered at temperatures of 30, 40, 50, 60, and 70 °C, respectively (Guenzi and Beard, 1976).

Soil moisture content plays an important role in influencing microbial degradation of pesticides. At low water content, inhibition of microbial growth is caused by inadequate water activity. At high moisture content, diffusion of air into the soil is reduced and microbial activity may become oxygen limited (Wise and Trantolo, 1994). Walker (1974) reported half lives of 54, 63, and 90 days with soil moisture contents of 10.0, 7.5, and 3.5%, respectively, at 28°C for napropamide (2-(*a*-naphthoxy)-*N,N*-diethylpropionamide). Similarly, Choi et al. (1988) determined half life values for DCPA (dimethyl tetrachloroterephthalate) to be 49, 33 and 31 d for low (0.1 kg H₂O kg⁻¹ soil), medium (0.2 kg H₂O kg⁻¹ soil), and high soil moisture levels (0.4 kg H₂O kg⁻¹ soil), respectively. Little difference in DCPA degradation was

seen between medium and high soil moisture contents, suggesting soil moisture was positively correlated with degradation rate, but only within a certain moisture range. The authors suggested that the moisture content of the medium soil moisture treatment may have been just above, or close to, the soil moisture content that supported an optimum population of DCPA degrading microorganisms. In general, the presence of water favors pesticide diffusion and subsequent sorption into hydrophilic sites within soil aggregates (Roy et al., 2000). Reduced soil moisture may hinder molecule transport to biodegrading soil microbes, thus reducing pesticide availability to microbes.

Soil organic carbon is an important soil constituent that frequently attenuates microbial degradation. Pesticide degradation rates have been found to be positively correlated to soil organic carbon as well as to microbial plate counts (Foster and McKercher, 1973; Veeh et al., 1996). Veeh et al. (1996) observed that the average half life values of 2,4-D at 24 °C were 2.5, 6, and 11 days for agricultural soils taken from depths of 0-30, 30-60, 60-120 cm that had averaged organic carbon contents of 10.4, 7.1, and 4.6 g kg⁻¹, respectively. There was a strong linear correlation ($r^2 = 0.94$, $n = 6$) between 2,4-D half life and percent soil organic carbon. Bacteria numbers obtained using plate counts in the same study decreased with soil depth and a positive correlation between bacteria numbers (represented by colony forming units) and percent organic C was observed. Grigera et al. (2006) reported significant correlations between total carbon and the bacterial and actinomycetes microbial pools, with the coefficients being 0.73 and 0.72 (both significant at the 0.01 level), respectively, in a composite agricultural soil sample collected at the 0- to 15-cm depth. Soil organic

matter is closely associated with microbial activity as it serves as substrates and energy sources for soil microbes (Magdoff and Weil, 2004).

Soil pH represents a major determinant of soil microbial distribution and activity as microbes have different pH requirements (Elsas et al., 2006). Singh et al. (2003) observed a strong relationship between chlorpyrifos [O,O-diethyl-O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate] degradation rate and soil pH. Chlorpyrifos half lives were 256, 58, 35, 16 and 16 days at soil pH values of 4.7, 5.7, 6.7, 7.7, and 8.4, respectively. The authors suggested that chlorpyrifos is degraded by non-specific and non-inducible enzyme systems produced in high pH soils.

2.2.3. Bioavailability of pesticides

While many factors influence pesticide degradation in soil or other porous media, sorption is recognized as a key process regulating pesticide degradation (van Genuchten and Wagenet, 1989; Scow, 1993; Shelton and Doherty, 1997). Adsorption of a chemical by soil reduces its availability to microbial uptake and thus reduces its degradation by microorganisms (Ainsworth et al., 1993).

Guo et al. (1999) studied adsorption kinetics and the resulting degradation of aldicarb [2-methyl-2-(methylthio) propionaldehyde *o*-(methylcarbamoyl) oxim]. Soil adsorption of aldicarb was modified by adding various amounts of activated carbon to soil. Activated carbon is a form of carbon processed to make it extremely porous. It has enhanced surface area available for adsorption. The addition of 0 to 5000 $\mu\text{g g}^{-1}$ of activated charcoal increased the adsorption coefficient of aldicarb from 0.36 to 47.4 mL g^{-1} . The degradation rate constant for aldicarb decreased from 0.0636 d^{-1} in

the absence of activated carbon to 0.0022 d^{-1} when the maximum amount of activated carbon was added. Activated carbon had no effect on overall soil microbial activity leading the authors to conclude that the decrease in degradation was primarily due to increased adsorption of aldicarb to activated carbon.

Farenhorst et al. (2008) compared 2,4-dichlorophenoxyacetic acid mineralization in well drained and imperfectly drained soil conditions. Samples were obtained from various spots under each of these two drainage conditions on a sloped agricultural field. The average percent soil organic carbon content and sorption coefficient (K_d) were 15.9 g kg^{-1} and 2.37 L kg^{-1} in well drained soils while being 29.9 g kg^{-1} and 3.24 L kg^{-1} in the poorly drained soils. The mineralization rate constants of 2,4-dichlorophenoxyacetic acid were 0.11 and $0.05 \text{ (day}^{-1}\text{)}$ in well drained and imperfectly drained soils, respectively. The differences observed between soil organic carbon content, sorption coefficient, and mineralization rate constants were all significant at the 0.05 level. The authors attributed the reduced 2,4-dichlorophenoxyacetic acid mineralization in imperfectly drained soils to their greater soil organic carbon content and increased 2,4-dichlorophenoxyacetic acid sorption, which limited the bioavailability of 2,4-dichlorophenoxyacetic acid for degradation.

Biodegradation of organic chemicals is generally believed to occur primarily in the liquid phase of the soil. This means adsorbed pesticides are not directly available to microbes (van Genuchten and Wagenet, 1989). Ogram et al. (1985) proposed three mathematical sorption-degradation models that described various possible effects of sorption on 2,4-dichlorophenoxyacetic acid degradation in soils. Validity of the models was tested by fitting the models to results of a series of controlled laboratory

experiments. The only model that worked indicated that 2,4-dichlorophenoxyacetic acid degradation could occur only in soil solution and was carried out by microbes present both in the soil solution and on soil surfaces. In other words, sorbed 2,4-dichlorophenoxyacetic acid was completely protected from biological degradation and only dissolved 2,4-dichlorophenoxyacetic acid in soil solution was available to degradation.

Guo et al. (1999) examined effects of adsorption on kinetics of aldicarb degradation. They used a silt loam that was amended with various amounts of activated carbon, resulting in five soils that each had a different adsorption coefficient. Degradation rate constants of aldicarb were estimated from first-order degradation of aldicarb in these five soils through a batch incubation experiment. By plotting degradation rate constants against the adsorption coefficient of each soil, a nonlinear regression was obtained that allowed Guo et al. (1999) to estimate that the degradation rate constant was 0.1228 and 0.0019 d⁻¹, respectively, for the liquid and sorbed phases of aldicarb. Degradation declined when more aldicarb was partitioned into the sorbed phase, as indicated by an increase in adsorption coefficient. Conversely, Park et al. (2001) suggested that pesticide desorption to the bulk solution is not prerequisite to degradation, and that the sorbed substrate may be available for degradation. By modeling the kinetics of sorbed-phase 2,4-dichlorophenoxyacetic acid in a silica-slurry system inoculated with a 2,4-D-degrading *Flavobacterium* sp. strain, Park et al. (2001) found that although degradation would occur primarily in the soil solution, it may not cease completely on the sorbed chemicals. The relative contributions of the liquid and sorbed phases to total degradation were dependent on

both the adsorption coefficient and the relative degradation rate constants for the dissolved and sorbed chemicals (Park et al., 2001). When pesticide sorption was separated into soluble phase, weakly and strongly sorbed phases, the strongly sorbed fraction was regarded as a pesticide reservoir that regularly provided pesticide to the weakly sorbed phase, and then, liquid phase, respectively (Saffih-Hdadi et al., 2006).

Bioavailability of sorbed organic chemicals in soils is linked to the characteristics of the sorbent and substrate, and is associated with the microorganisms that use the chemical as a substrate and energy source (Park et al., 2001). Guerin and Boyd (1992) observed drastically different bioavailability of soil-sorbed naphthalene to two bacterial species under investigation. They suggested the bioavailability of naphthalene may be organism-specific, thus it is inappropriate to make generalizations regarding the bioavailability of sorbed substrates.

Adsorption and degradation are not fixed properties of a pesticide (Dyson et al., 2002). The effect of sorption on degradation depends on many factors, including soil physical and microbial characteristics and the properties of the chemical being evaluated (Beulke et al., 2005). Soil organic matter is considered to be the single most important soil constituent influencing pesticide sorption in soils (Wauchope et al., 2002; Farenhorst, 2006). In soils, pesticides are initially and predominately sorbed to organic matter that coats soil particles (Park et al., 2003). Generally, the lower the water solubility of a chemical and the higher the amount of organic carbon in the soil, the greater the sorption of a hydrophobic compound (Alexander, 1999).

While sorption generally slows pesticide biodegradation, there are certain occasions where sorption may actually accelerate pesticide biodegradation. One

frequently cited example is the increased residence time of pesticides in the root zone where microbial activity is very high at or near the particle surfaces as compared to the underlying stratum (Hance, 1988). Reduced concentrations of oxygen and nutrients can occur inside soil particles due to competition from microorganisms at the surface (Beulke et al., 2005). Thus sorption may provide an opportunity for extended exposure to degrading microorganisms.

2.3. MICROBIAL ACTIVITY AND PESTICIDE DECAY IN TURF

2.3.1. Methods used to assess microbial activity in turf

Microorganisms play various roles in the growth, disease occurrence, organic matter decomposition, and in environmental fate of nutrients and pesticides applied to turf. A number of methods have been used to assess microbial activity in turf systems.

Fluorescein diacetate (FDA) hydrolysis assay is a means to estimate the general level of microbial activity in environmental samples with the enzyme activity of microbial populations. The assay is non-specific because it is sensitive to the activity of several enzyme classes including lipases, esterases, and proteases. The activity of these enzymes results in the hydrolytic cleavage of FDA (colorless) into fluorescein (fluorescent yellow-green). The intensity of the resulting yellow-green color is indicative of the amount of enzymatic cleavage of the FDA molecule and the overall enzymatic activity in the sample. Quantification of enzyme activity is performed by assessing the intensity of color formation using spectrophotometry. This method does not require the isolation of specific microorganisms and has been successfully applied to turfgrass research (Davis and Dernoeden, 2002). Using this method, Davis and

Dernoeden (2002) investigated the general soil microbial activity present in a fairway height maintained creeping bentgrass (*Agrostis stolonifera* L.) that received annual applications of nine nitrogen sources for seven years. They found that none of the nitrogen sources consistently induced enhanced soil microbial activity when microbial activity of soil was measured in the last two years of the study.

Mancino and Pepper (1992) used an agar-plate method to enumerate total aerobic culturable bacteria populations in a turf soil irrigated with secondary sewage effluent vs. potable water. This method is based on direct total microbial count obtained from bacterial growth of an environmental sample on the plate count agar. The plate count agar is a non selective microbiological growth medium and it enables monitoring of total bacterial growth. Total aerobic population ranged from a low of 2.5×10^5 to a high of 7.9×10^9 CFU (colony-forming unit) g^{-1} dry soil when turf was irrigated with effluent water over a ~3-yr period. The total aerobic bacteria populations were similar between the irrigation sources indicating that these microbes were not promoted or inhibited by the use of secondarily treated wastewater. Similarly, Smiley and Fowler (1986) used the agar-plate method to study select microbial populations in thatch and soil in long-term fungicide treated turf sites. Enumerations were made of total bacteria, actinomycetes and fungi, and of *Bacillus*, *Pseudomonas*, and *Fusarium* spp. None of the fungicides significantly altered thatch microbial composition when microorganisms were evaluated to the group and/or genus level over two seasons.

Measurement of the evolution of CO₂ from soil is a common methodology used to assess general microbial activity (Grant and Rochette, 1994; Osborne et al., 2006).

Martin and Beard (1975) used this approach to examine variations in thatch microbial activity within a red fescue (*Festuca rubra* L.) thatch that was amended with different chemical additives. Using this method, thatch samples were sealed in test tubes and incubated in a controlled environment chamber and CO₂ gas evolved was determined by gas chromatography. Results indicated that this technique can be useful in studying microbial decomposition of thatch in turf.

Turfgrass soil microbial biomass can be estimated by quantifying phospholipid extracted from soils. Phospholipids are key components of the cell membrane and they undergo rapid degradation once a cell dies. The amount of phospholipid in a soil sample can serve as a direct biochemical indication of the size of the active biomass (White et al., 1996). Compared to cultural methods such as plate counts that require growth on selective media, phospholipid content measurement is a direct approach. Using this method, Acosta-Martinez et al. (1999) examined the microbial biomass of a perennial ryegrass soil as it was influenced by different N fertility regimes. Similarly, Kerek et al. (2002) used the lipid phosphate extraction method to determine microbial biomass on golf greens.

2.3.2. Methods used to assess pesticide decay in turf

Pesticide decay in turf can be examined by conducting field based degradation studies, where pesticides are applied to turf on-site and samples are taken periodically to quantify the concentration of chemical residue. Gardner and Branham (2001) examined the dissipation of mefenoxam [*N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl)-*D*-alaninemethyl ester] and propiconazole (1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-

dioxolan-2-yl]methyl]-1*H*-1,2,4-triazole) under field conditions on a creeping bentgrass. They constructed sampling cylinders from polyvinylchloride (PVC) pipes that were inserted into the plot prior to on-site pesticide application. The sampling cylinders were removed from field at select dates during their 64-day study period and pesticide residue concentration determined. Mefenoxam had a 5.5 day half life and propiconazole had a 13.5 day half life.

Nash and Beall (1980) conducted turf pesticide fate studies with microagroecosystem chambers, where they examined 2,4-D in a Kentucky bluegrass (*Poa pratensis* L.). Their system consisted of a rectangular glass chamber constructed of glass plates. There were large holes drilled on the glass plates for air intake and outlet and small holes for irrigation and drainage. Sliding glass doors were installed on one side of the chamber to permit servicing. Established bluegrass turf sod was placed on top of soil that was already in the chamber and the pesticides were applied. Samples were collected and analyzed on select dates for radiolabeled pesticide residue concentrations in the air, grass clippings, soil cores, and leachate from a sprinkle irrigation inside the chamber. In their study, 2,4-D had a 10 day half life in soil and a 14 day half life in the air. This method allowed for total account of pesticide losses in or on plants, soil, water, and air after application.

Laboratory based incubation studies are commonly used methods to determine degradation characteristics of turf-applied pesticides. Using this method, Hurto et al. (1979) found that the breakdown of benefin (*N*-butyl-*N*-ethyl- α,α,α -trifluoro-2,6-dinitro-*p*-toluidine) was minimal in a Kentucky bluegrass soil but substantial in thatch. An incubation study by Wu et al. (2002) showed that chlorpyrifos [O,O-

diethyl-O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate] had a half life of 119.5, 247.6, and 266.6 days, respectively, in the soil, thatch, and mat layers of a creeping bentgrass putting green turf.

Laboratory based pesticide degradation incubation studies are more easily interpreted than field based pesticide dissipation studies in that results are obtained from controlled laboratory conditions. Incubation investigations permit differentiating between microbial degradation and chemical decomposition of a pesticide by comparing sterilized sample pesticide losses with non-sterilized sample pesticide losses (Suzuki et al., 1996). In turf, published reports employing the use of sterilized sample material to evaluate the degradation of pesticides in thatch appear to be limited to a single study investigating the biodegradation of dicamba (Roy et al., 2001). In this study near complete breakdown of dicamba (i.e. > 90%) was observed in the non-sterilized thatch samples by the end of a 100 day incubation period. Dicamba levels in the sterilized thatch samples remained fairly constant over the evaluation period indicating volatilization and chemical degradation losses of dicamba are minimal in thatch.

2.3.3. Pesticide decay in turf

Microbial decay is an important route of pesticide dissipation from turf. A recent review by Magri and Haith (2009) suggested that pesticide dissipation is considerably faster in well established turf than in soil. In such cases, enhanced microbial activity is frequently cited as the basis for faster rates of pesticide dissipation seen in turf field studies, especially since large, highly active, and

adaptable microbial populations reside in thatch (Branham et al., 1993; Horst et al., 1996; Roy et al., 2001). While turf field studies often can only determine pesticide dissipation, laboratory incubation studies, on the other hand, can more specifically examine pesticide degradation in turf, especially by comparing pesticide degradation in thatch and soil.

Thatch as a supporting medium provides a more suitable environment for microbes than the underlying soil (Raturi et al., 2004). This may explain why the degradation rate of dicamba in a batch experiment in a Kentucky bluegrass (*Poa pratensis* L.) thatch was 5.9 to 8.4 times faster than in the soil (Roy et al., 2001). Published studies examining the degradation of pesticides in both thatch and soil are relatively few in number. A compilation of literature comparing degradation half lives in thatch and soil is listed in Table 2.1. The results of this compilation, while somewhat tenuous because of the limited number of pesticides examined, suggest that pesticide degradation rate in thatch is not always greater than it is in the underlying soil. Degradation of pesticides with high sorption potential appears to proceed more slowly in thatch than in the underlying soil. Conversely, these laboratory studies appear to confirm more rapid breakdown of a pesticide will occur in thatch than in soil if the pesticide has low sorption potential. The comparative characteristics of pesticide degradation in thatch versus soil as seen in Table 2.1 appear to suggest that the greater pool of microbial activity present in thatch is largely ineffective in hastening the degradation of pesticides that readily sorb to thatch and soil. This is consistent with the principal that as the proportion of pesticide residing in the liquid phase of medium declines, the microorganism community within the medium has a

Table 2.1. Pesticide laboratory incubation half lives for thatch and the underlying soil listed in order of the likely mobility of each pesticide.

Pesticide	Koc [†]	Half Life (day)		Reference
		Thatch	Soil	
Chlorpyrifos	6070	248	120	Wu et al., 2002a
Chloroneb	1650	8.7	2.7	Frederick et al., 1994
Chlorothalonil	1380	2.2	2.7	Wu et al., 2002b
Triadimefon	300	8.7	4.3	Frederick et al., 1994
Vinclozolin	100	7.7	0.8	Frederick et al., 1994
Metalaxyl	50	110	165	Wu et al., 2002b
Trichlorfon	10	1	0.5	Wu et al., 2002b
Dicamba*	1	11	75	Roy et al., 2001

* Average half life for four different environmental conditions.

† The normalized sorption coefficient (Koc) is defined as the equilibrium liquid to solid phase distribution coefficient [$K_d = (\text{mg pesticide sorbed to medium} / \text{kg medium}) / (\text{mg pesticide in solution} / \text{L solution})$] of a pesticide within a medium, divided by the fraction of organic carbon (%OC/100) present within the medium. The normalized sorption coefficient is a relative indicator of the proportion of pesticide initially introduced into the liquid phase of a medium that is sorbed to the organic carbon present in the medium.

reduced ability to utilize the pesticide as a substrate (van Genuchten and Wagenet, 1989).

2.3.4. Microbial decay of 2,4-D, flutolanil and chlorpyrifos

i) 2,4-D

The chlorinated phenoxy compound 2,4-dichlorophenoxyacetic acid is a common systemic herbicide used to control broadleaf weeds in agricultural and nonagricultural settings. Major sites of use include pasture and rangeland, residential lawns, roadways, and cropland. According to the EPA, annual domestic usage of this herbicide is approximately 20.9 million kg, with 13.6 million kg (66%) used by agriculture and 7.3 million kg (34%) used in non-agriculture settings. The application rates per application and rates per year are generally less than 1.7 kg acid equivalents per ha and 2.3 kg a.e./ha, respectively (EPA 2,4-D Reregistration Eligibility Decision Facts, 2005). 2,4-dichlorophenoxyacetic acid is made in various forms including acid, ester, and amine salt. Esters are formed by reacting the parent acid with an alcohol, while amine salts are formed when the parent acid is reacted with an amine. This herbicide is applied primarily as either an amine salt or a low-volatile ester in general agriculture (Waite et al., 2002). Similarly in turf, salt and ester forms of 2,4-D are the most commonly used forms. 2,4-dichlorophenoxyacetic acid has a water solubility of 900 mg/L, an average K_{oc} of 20 and a general half life of 10 days in agricultural soils (ARS PPD; USDA-ARS, 2006). Microbial degradation is considered to be the major route in the breakdown of 2,4-D in soil (Parker and Doxtader, 1983; Ou, 1984). A primary 2,4-D degradation metabolite in moist nonsterile soils is 2,4-dichlorophenol

(2,4-DCP), which can further undergo biological methylation to 2,4-dichloroanisole (2,4-DCA) (Smith, 1985; Smith and Aubin, 1991).

Various soil factors can affect the microbial breakdown of 2,4-D. Parker and Doxtader (1983) observed that 2,4-D degradation in soils with moisture retentions of 0.05 and 0.1 MPa was minimum. Under these two moisture conditions there was little or no activity of 2,4-D degrading microorganisms in the soils due to decreased water availability. The same researchers also indentified 27 °C to be an optimum temperature for 2,4-D degradation in a sandy loam. 2,4-dichlorophenoxyacetic acid degradation occurred by a slow, first-order reaction (slow phase) that was followed by a rapid, first-order reaction (fast phase) at 27°C. As temperatures went above 27°C, no fast phase of degradation was observed. Veeh et al. (1996) studied 2,4-D degradation in two agricultural soils and found a strong linear correlation between 2,4-D half life and percent soil organic carbon ($r^2 = 0.94$, $n = 6$). Average half life values of 2,4-D in the two soils were 2.5, 6, and 11 days at soil depths of 0-30, 30-60, 60-120 cm that had averaged organic carbon contents of 10.4, 7.1, and 4.6 g kg⁻¹, respectively. Soil factors such as moisture, temperature, and organic matter content have an effect on microbial activity in the soil and subsequently affect microbial breakdown of the pesticide.

The herbicide 2,4-D had a 10 day half life in a Kentucky bluegrass soil in a microagroecosystem chamber (Nash and Beall, 1980). In a green house study, 2,4-D residues on or in a Kentucky bluegrass declined from 92% of that originally applied, to less than 66% by day 15; and average 2,4- D dissipation half life was 23.7 in their system (Thompson et al., 1984). Starrett et al. (2000) reported 2,4-D recovery after

their 28-day field study to be 3.12% when residues were averaged across thatch/mat, soil, and the leachate; the averaged 2,4-D dissipation half life was 5 days.

ii) Flutolanil

Flutolanil [N-(3-(1-methylethoxy)phenyl)-2-(trifluoromethyl)benzamide] is an organic, systemic, acropetalpenetrant fungicide in the carboximide chemical class. It is registered in the United States for use on certain turfgrasses for the control of such diseases as fairy ring (*Tricholoma sordidum* Fr.), gray leaf spot (*Pyricularia grisea*), Pythium blight (*Pythium* spp.), rhizoctonia brown patch (*Rhizoctonia solani*), and rust diseases (*Puccinia* spp.) (Beard, 2005). Flutolanil has a Koc of 418, indicating it's moderately mobile in soil (Briggs, 1981). It is somewhat hydrophobic with a water solubility of 8.0 mg L⁻¹ (IUPAC, 2009). The primary degradation metabolite of flutolanil under aerobic soil condition is desisopropyl flutolanil [N-(3-hydroxyphenyl)-2-(trifluoromethyl)benzamide] (Dewhurst, 2002).

There are relatively few published investigations that have examined flutolanil dissipation in the turf environment. Suzuki et al. (1998) examined flutolanil degradation by incubating a fairway Zoysiagrass (*Zoysia japonica* Steudel) soil in aerobic and anaerobic environments. The half life for flutolanil was 336 days under aerobic conditions and 361 days under anaerobic conditions. Suzuki and Otani (2004) compared flutolanil degradation in two agricultural soils. The soils were a loam soil containing 66 g kg⁻¹ total carbon and a clay loam soil containing 22 g kg⁻¹ total carbon. Both sites were used to grow barley and other crops. Flutolanil degradation half lives were 82.5 and 76.4 days, respectively, for the loam and clay loam soil.

Uchida et al. (1983) detected five degradation products of flutolanil in upland agricultural soils while only three of the five products were detected in flooded soils, indicating flutolanil degradation proceeded more extensively in the more aerated upland soils.

iii) Chlorpyrifos

Chlorpyrifos (*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridylphosphorothioate) is a crystalline pyridine insecticide used to control foliage and soil-borne insect pests on a variety of food and feed crops. First introduced in 1965 (Singh and Walker, 2006), chlorpyrifos is one of the most widely used organophosphate insecticides in the U.S. and, until 2000 when nearly all residential uses were cancelled, was one of the major insecticides used in residential settings (EPA, 2006). In 2002, the U.S. EPA required that the label rate for the use of chlorpyrifos on golf courses in the U.S. be reduced from 4.48 kg ha⁻¹ to 1.12 kg ha⁻¹ (EPA, 2006). In turf, chlorpyrifos can control insect pests such as Japanese beetles (*Popillia japonica*) and cutworms (*Agrotis ipsilon*). Chlorpyrifos can be degraded by a combination of chemical hydrolysis and microbial degradation, with the primary metabolite being 3,5,6-trichloro-2-pyridinol (Smith et al., 1967). Microbial degradation of chlorpyrifos can be significant in various soils as indicated by significantly faster degradation rates in non-sterile versus sterile soil (Howard and Michalenko, 1991; Singh et al., 2003). Chlorpyrifos has a low water solubility (1.2 mg L⁻¹) and a high K_{oc} of 6070 (ARS PPD; USDA-ARS, 2006).

The half life of chlorpyrifos has been found to range 4 to 139 days in agricultural soils (ARS PPD; USDA-ARS, 2006). The wide variation in half life is

due to different soil conditions. Cink and Coats (1993) observed that the highest percent of chlorpyrifos mineralization occurred in soil maintained near field capacity (0.30 bar) while the lowest percentage occurred in soil maintained under the driest condition (3.0 bar). Singh et al. (2006) used chlorpyrifos-degrading bacterial isolates to find that the optimum temperature for chlorpyrifos degradation is between 15 and 35 °C. In turf, Wu et al. (2002) observed that chlorpyrifos degradation is slower in thatch (half life = 248 days) than in soil (half life = 120 days).

2.4. TURF CULTIVATION PRACTICES

2.4.1. Vertical mowing and hollow tine cultivation

Soil compaction and excessive thatch buildup can lead to deterioration in turf quality. Unlike conventional crops, turf areas cannot be plowed to restore the vitality of turf. Thus mechanical practices such as vertical mowing and hollow tine core cultivation are commonly used to improve turf quality. These practices are performed on a curative and preventive basis to reduce excessive soil compaction and thatch buildup.

Vertical mowing is performed by a vertical mower. A vertical mower has evenly spaced blades that revolve perpendicularly to the turf that slice stolons and rhizomes and mechanically removes decaying plant materials. Vertical mowing promotes the lateral and vertical growth of turf (Beard, 1973) and creates channels that facilitate moisture and oxygen movement into the root zone.

Hollow tine core cultivation is a practice by which a core cultivator is used to extract small soil cores from the turf. A hollow tine cultivator vertically inserts

hollow tines into the soil, and selectively removes soil plugs 0.5 to 2.0 cm in diameter and 5 to 10 cm deep, depending on machine type and weight, and soil texture and moisture. The removal of cores aids in the movement of air, water, and nutrients into and through the cultivated portion of the soil (Beard, 1973). Extracted soil cores can either be removed and the opened holes filled with sand or soil, or the soil cores can be reincorporated to turf. Hollow tine core cultivation physically and selectively removes thatch as soil cores are removed.

2.4.2. Modification of thatch edaphic properties by cultivation practices

i) Bulk density

Repeated hollow tine core cultivation, with the cores returned, introduces soil into the thatch layer. When soil was reincorporated into a creeping bentgrass putting green following hollow tine core cultivation, thatch bulk density increased from 0.24 mg m^{-3} to 0.61 mg m^{-3} over a 3-year trial period, when compared with the bulk density of untreated control plots (Murphy et al., 1993). Likewise, Danneberger and Turgeon (1986) found that thatch bulk density was increased by incorporating soil cores removed from hollow tine cultivation in their field studies on a creeping bentgrass green and a Kentucky bluegrass lawn turf.

ii) Thatch organic matter content

Vertical mowing and core cultivation can alter the amount and quality of organic matter present in thatch. Danneberger and Turgeon (1986) found that thatch edaphic properties were altered by soil cultivation and the incorporation of removed

soil cores. Cultivation treatments in their study included core cultivation once or three times a year with soil cores reincorporated or removed, vertical mowing, and combinations of these treatments. Thatch ash content decreased as the intensity of core cultivation increased from 1 to 3 times per year (thatch ash is the non-combustible mineral residue left after thatch organic matter is burnt off). When soil cores were reincorporated, ash content decreased compared to treatments where the cores were removed. Making three passes of over the turf with a hollow tine cultivator increased the mineral content of the thatch from 18.5% to 66.1% when cores were returned and when samples were collected six weeks after cultivation. Vertical mowing treatments significantly affected ash content in the same way as coring. Passing a verticutter over the same turf twice reduced the organic matter content of the thatch by 13% when samples were collected six weeks after cultivation.

Murphy et al. (1993) examined the effect of hollow and solid tine cultivation on the organic matter content and organic matter fraction of thatch in a 3-year study conducted on a creeping bentgrass putting green. Hollow tine cultivation increased thatch/mat organic matter by 150 g m^{-2} compared to solid tine cultivation, when averaged over noncompacted and compacted plots. The authors attributed the difference in organic matter to the greater removal of thatch/mat by hollow tine cultivation, which may have induced some additional thatch/mat growth to compensate for the greater amount of material removal. The fraction of organic matter (kg kg^{-1}) in thatch decreased with hollow tine cultivation, when compared with solid tine cultivation. The addition of soil to thatch with hollow tine cultivation was

primarily responsible for the lowered fraction of organic matter in thatch (Murphy et al., 1993).

iii) Microbial activity

The effects of vertical mowing and hollow tine cultivation on thatch microbial activity are not well understood. Past efforts to quantify microbial activity in turf have largely focused on determining the total populations of fungi and bacteria, or total microbial biomass present in sand-based golf greens. In such situations increasing microbial activity has largely been attributed to expanding pools of decomposing plant material (Kerek et al., 2002) and to conditions that favor rooting activity (Bigelow et al., 2002). Kerek et al. (2002) found in putting greens that soil microbial biomass generally increased as greens aged. The increase was primarily attributed to the accumulation of particulate organic matter in the soil.

Bigelow et al. (2002) reported that resident or introduced microorganisms could increase rapidly in sand-based rootzones during initial turfgrass establishment; and that these rootzones could sustain a large and diverse microbial community. Within the first 6 months of establishment, microbial populations increased from 10^6 to $>10^8$ cfu g⁻¹ dry soil in newly constructed sand-based rootzones. The same study found that sand rootzone amendments did not significantly affect microbial populations. The authors suggested that the presence of a young and actively growing turfgrass root system may have created opportunities for the proliferation of soil organisms, independent of any rootzone amendment. In a mature creeping bentgrass putting green (>5 yr old), Mancino et al. (1993) reported a relatively large microbial

population of $>10^7$ cfu g^{-1} soil, similar to populations found in some native soils. The authors also found much greater microbial population in thatch of the sand-based rootzone, compared with the underlying rootzone.

Structural alterations in thatch arising from the introduction of soil into the media may alter microbial activity by providing a more or less favorable environment for moisture retention and rooting activity. Increased moisture availability in the absence of hypoxic conditions generally favors microbial activity (Orchard et al., 1983). Although there is a dearth of information on the comparative moisture retention properties of cultivated and uncultivated thatch, it has been hypothesized that the addition or incorporation of soil into thatch enhances microbial activity by improving the moisture retention properties of thatch (Hurto et al., 1980).

Berndt (2008) suggested that increasing the surface area of thatch could help increase microbial growth efficiency of thatch. Cultivation practices such as hollow tine cultivation and vertical mowing have a grinding effect on thatch and thus may increase the surface area of thatch to some extent. The increase in surface area of organic material brought about by hollow tine cultivation and vertical mowing may help convert thatch organic carbon into thatch microbial biomass and reduce turnover times of organic carbon in thatch because of enhanced microbial growth efficiency.

To date, no published research has been identified that examined the comparative microbial activity of thatch and soil when the turf was subjected to vertical mowing or hollow tine cultivation.

CHAPTER III.

EFFECT OF CULTIVATION ON THATCH AND SOIL MICROBIAL ACTIVITY

Turfgrass is the predominant feature found in urban and suburban landscapes. In mature turf an organically rich zone often develops immediately above the soil surface when plant biomass production exceeds organic matter decomposition. This zone, which is called thatch, consists of living stems and roots as well as stems, roots and leaf sheaths in various states of decay. The presence of large amounts of partially decomposed plant materials in turfgrass thatch typically supports higher levels of microbial activity than the underlying soil. Thatch contains 40 to 1600 times as many bacteria, 500 to 600 times as many fungi, and up to 100 times as many actinomycetes (Mancino et al., 1993) as soil. Thatch also has an average of 10 times greater total microbial biomass carbon than the underlying soil (Raturi et al., 2004). The presence of high levels of microbial activity in thatch is considered to be a favorable attribute of the medium because it enhances the biodegradation of synthetic organic compounds in turf (Beard and Green, 1994). The high microbial activity in thatch is frequently used as an explanation to account for accelerated rates of pesticide degradation seen in turf compared to aerobic soils or other crop systems (Horst et al., 1996; Gardner et al., 2000; Gardner and Branham, 2001; Magri and Haith, 2009). Microbial activity in turf also is associated with processes important for maintaining

turf health, such as nutrient supply and cycling, disease occurrence, and disease suppression (Turgeon, 2001; Kerek et al., 2002).

Soil compaction and excessive thatch buildup can lead to deterioration in turf quality. Mechanical practices such as hollow tine core cultivation and vertical mowing are commonly employed to improve soil physical properties and to restore turf quality. These practices can alter the amount and quality of organic matter present in thatch, which may increase or decrease microbial activity within this medium. Vertical mowing can remove significant quantities of the decaying plant material within thatch, which may favor reduced levels of microbial activity in recently cultivated thatch compared with uncultivated thatch. Conversely, Berndt (2008) has proposed that vertical mowing, by increasing the surface area of decaying organic matter, will stimulate microbial activity in thatch. Structural alterations in thatch arising from the introduction of soil into the medium may favor enhanced microorganism activity in thatch (Williams and McCarty, 2005; Brandt, 2008). Hollow tine cultivation introduces soil into thatch when the cores are broken up and worked into the turf canopy (Danneberger and Turgeon, 1986; Murphy et al, 1993). Although there is a dearth of information on the comparative moisture retention properties of cultivated and uncultivated thatch, it has been hypothesized that the addition or incorporation of soil into thatch will enhance microbial activity within thatch by improving its moisture retention properties of thatch (Hurto et al., 1980).

Past efforts to quantify microbial activity in turf have largely focused on determining the total populations of fungi and bacteria, or total microbial biomass present in sand-based golf greens (Mancino et al., 1993; Kerek et al., 2002; Bigelow

et al., 2002). In such situations, increasing microbial activity has largely been attributed to expanding pools of decomposing plant material (Kerek et al., 2002). In soils microbial pools often are positively associated with the organic matter present in the soil due to its role as substrates and energy sources for microbes (Magdoff and Weil, 2004; Grigera et al., 2006). Moisture is another factor that can influence microbial activity (Schjønning et al., 2003). In soils, low water content can inhibit microbial growth because of inadequate water activity. In soils having high soil moisture levels microbial activity declines due to a general reduction in oxygen exchange rate within the soil (Wise and Trantolo, 1994). The effect of moisture on the microbial activity of thatch however is not well known. While organic matter can be seen as more of an inherent property of thatch and soil, moisture content of each media is more likely to be altered due to precipitation or irrigation practices. Although both organic matter and moisture can influence microbial activity, the importance of moisture relative to the amount of organic matter present in thatch and soil on microbial activity in each media is not clear. The objectives of this study are to 1) determine the effect of hollow tine cultivation and vertical mowing on the microbial activity of thatch and soil and to 2) determine the strength of relationships that exist between thatch and soil moisture content and microbial activity, and thatch and soil organic matter content and microbial activity. The objectives were evaluated by examining thatch and soil microbial activity for an eight week period following hollow tine core cultivation and vertical mowing of two turfgrass sites.

MATERIALS AND METHODS

Site Locations

The effect of turfgrass cultivation practices on thatch and soil microbial activity was examined at two locations in the spring and summer of 2008. The first site was a stand of 'L-93' creeping bentgrass (*Agrostis stolonifera* L.) located on 3.5% hillside slope at the University of Maryland Paint Branch Turfgrass Research Facility in College Park, MD. This site was seeded in May of 2004. The soil at the site was mapped as Keyport silt loam (fine, mixed, semiactive, mesic Aquic Hapludult). The surface 2.5 cm of soil contained 34.3% sand, 46.6% silt, 19.1% clay, 48 g kg⁻¹ organic matter, and had a pH of 5.2. The thatch at this site contained 458 g kg⁻¹ organic matter and had a pH of 5.9. Visual inspection of the thatch revealed it consisted of a tightly matted network of live and partially decomposed fine textured stolons intermingled with a mass of white and tan colored roots. Prior to this investigation the block was maintained as a simulated golf turf fairway for the purpose of conducting nutrient and pesticide runoff investigations. The most recent cultivation practice performed on the site prior to this investigation was two passes of a vertical mower on 28 Mar. 2007 with the blades of the vertical mower set to penetrate the surface 0.25 cm of soil.

The second site was a 14 year old sward of Meyer zoysiagrass (*Zoysia japonica* Steud.) that was located on 17th fairway of the South Course at Woodmont Country Club (WCC) in Rockville, MD. The soil at the site was mapped as Baile silt loam (fine-loamy, mixed, semiactive, mesic Typic Endoaquults). The surface 2.5 cm of soil contained 47.5% sand, 33.5% silt, 19.0% clay, 127 g kg⁻¹ organic matter, and

had a pH of 6.5. The thatch at this site contained 620 g kg⁻¹ organic matter and a pH of 4.9. The thatch consisted of a loose mass of live and partially decomposed coarse textured stolons intermixed with soil. Prior to this investigation the most recent cultivations imposed on the site included one pass of a slit aerifier set to penetrate the surface 5.0 cm of soil with no material removed in the summer of 2007.

Cultivation Treatments

Each site was divided into three blocks. Three cultivation treatments were imposed separately within 3.0 m x 3.0 m plots placed within each block. The cultivation treatments were imposed immediately after mowing the turf. Thatch thickness immediately before imposing the cultivation treatments ranged from 1.5 to 2.0 cm at the creeping bentgrass site and 0.9 to 1.2 cm at the zoysiagrass site. The three cultivation treatments were no cultivation (N), three passes over the plot with hollow tine cultivator with the cores returned (HT), and three vertical mower passes over the plot with the debris created by the treatment removed (VM). The HT cultivation treatment was imposed using a Jacobsen Lawnaire IV aerator (Jacobsen, a Textron Company, Charlotte, NC) equipped with 1.9 cm diameter tines. The aerator created a 9.5 cm x 18 cm hole pattern and was set to extract soil cores to a depth of 7 cm. The portion of surface area from which cores were pulled by the aerator was 5% (on area basis). A Bluebird EasyScape lawn comb (BlueBird Lawn Comber Company, Beatrice, NE) with a 5 cm spacing between blades, was used for vertical mowing, with the depth of penetration set to 4.0 cm. The three passes within each plot were executed in such a way that two of the passes were made along the two

perpendicular sides of the square and the third pass was made along the direction of the diagonal. Debris created by vertical mowing was removed from the plots using a leaf rake. A metal garden rake was used at the creeping bentgrass site to break up soil cores left on the turf canopy of the hollow tine cultivated plots. A level on rake was used at the zoysiagrass site to accomplish the same task.

Regular mowing at the creeping bentgrass site began on 1 Apr in 2008 with the turf being cut at a height of 1.25 cm once per week for two weeks. Thereafter, the turf was mowed two to three times per week. Cultivation treatments were imposed at this site on 16 April 2008. On 18 April the plot area was fertilized with 49 kg N ha⁻¹ using urea (46-0-0) with the fertilizer being watered in shortly after application. Partially pulverized soil cores remaining on the surface of the hollow tine cultivated plots were removed from surface of the creeping bentgrass when the plots were mowed on 23 April 2008. Regular mowing at zoysiagrass site began in mid-April in 2008 with the turf being mowed to 1.27 cm two times a week. Cultivation treatments were imposed at this site on 27 May 2008. On June 3, the plot area was fertilized with 49 kg N ha⁻¹ using urea (46-0-0) and the fertilizer watered in the same day.

Field Collections & Laboratory Processing

Thatch and soil were collected from both sites at approximately 2 week intervals for 8 weeks. Sampling began 13 to 15 days after imposing the cultivation treatments. Sample collection dates at the creeping bentgrass site were 2 May, 15 May, 30 May and 14 June (i.e. 15, 28, 43, and 58 days after cultivation). Sample collection dates at the zoysiagrass site were 10 June, 24 June, 7 July, and 24 July (i.e.

13, 27, 40 and 56 days after cultivation). At both sites, samples were collected by extracting six thatch + soil cores from each plot. A Mascaro Profile Sampler 18.0 cm deep, 7.6 cm wide and 1.3 cm thick (Turf-Tec International, Tallahassee, FL) was used to extract a cross section of the thatch and soil profile. The cores were driven to a depth sufficient to extract the thatch, plus 3 to 4 cm of soil to ensure 2.5 cm of soil was collected. Cores containing a cross section of verdure, thatch and soil profile were placed into plastic bags that were sealed and stored in a closed heavy-duty paper box and then transported to the lab immediately after all sample material had been collected from the field. Sample collection on most dates commenced between 6:00 and 7:00 hr, and was usually completed within 35 minutes.

Once samples were brought into the laboratory the verdure portion of the core was excised and then discarded. Thatch was separated from soil using a knife blade or scissors. A ruler was used to insure only the surface 2.5 cm of soil was collected for analysis. The remainder of soil extracted using the profiler was discarded. Field moist zoysiagrass thatch was passed through a 4 mm sieve after being separated from the underlying soil. Because of tightly matted character of the bentgrass thatch, this media could not be passed through 4 mm sieve in a field moist state. Partial homogenization of the bentgrass thatch collected from a single plot was achieved by cutting the thatch into numerous small pieces using scissors. Soil from each plot was passed through a 2 mm sieve after being separated from thatch. Sample materials were processed on a plot by plot basis to ensure the properties the sample material collected from a plot remained independent of all other plots throughout the sample processing and measurement procedures. The knife blade or scissor used to separate

verdure/thatch/soil was rinsed with ethyl alcohol and air dried between processing sample material collected from different plots.

Thatch and soil microbial activity

Thatch and soil microbial activity was determined by fluorescein diacetate (FDA) hydrolysis. This method does not require the isolation of specific microorganisms and has been successfully applied to turfgrass research (Davis and Dernoeden, 2002). Fluorescein diacetate is hydrolyzed by a number of enzymes made available by soil microorganisms. The product of this enzymatic reaction is fluorescein, which can be quantified by spectrophotometry. Determination of the rate of FDA hydrolysis has been proven to be an effective assessment of microbial activity (Schnrer and Rosswall, 1982).

Fluorescein diacetate hydrolysis was performed by first placing 5 g field moist soil, or 1 g field moist thatch, into a 250 mL Erlenmeyer flask. Twenty milliliters of 60 mM sodium phosphate buffer (pH = 7.6) was added to each flask, followed by the addition of 400 µg of FDA. The flasks were then shaken at room temperature for 1 hr on an orbital shaker operated at a speed of 90 rpm. At the end of the one hour, 20 mL of acetone was added to each flask. Thatch or soil residues were removed from the mixture by filtering the suspension through Whatman No. 1 filter paper. Filtrate in the amount of 1.5 mL was diluted with an equal volume phosphate buffer (1:1 vol/vol) after which the fluorescein concentration of the resulting solution was determined by spectrophotometrical absorbance at 500 nm (Spectronic® Genesys™ 2 Spectrophotometer, Golden Valley, MN). Absorbance blanks were also prepared to

compensate for absorbance by soluble components present in the thatch or soil. The absorbance blanks consisted of 5 g soil or 1 g thatch plus phosphate buffer but with no FDA added.

Standard reference curves of FDA hydrolysis were prepared for each cultivation treatment by mixing together thatch or soil from the plots receiving a specific cultivation treatment for each date sample material was collected. Six standard curves, three for thatch and three for soil, were generated on each collection date. The standard curves were prepared following the procedures outlined by Davis and Dernoeden (2002). A fluorescein diacetate stock solution having a concentration of 1.0 g/L was made by dissolving FDA in acetone. Additions of 0, 100, 200, 300, and 400 μ L of this stock solution were made to five separate 40 mL screw cap tubes, each of which contained 5 mL 60 mM sodium phosphate buffer solution (pH = 7.6). Tightly capped tubes were then incubated in a water bath maintained at 95 °C for 60 min to hydrolyze FDA. Upon cooling, the fluorescein and buffer mixture were transferred to a 250 mL Erlenmeyer flask that contained 5 g soil, or 1 g thatch, and an additional 15 mL of phosphate buffer. The flasks were incubated for 45 min at room temperature on a rotary shaker operated at 90 rpm, after which 20 mL of acetone was added to stop FDA hydrolysis. The samples were then poured to pass through Whatman No.1 filter paper and the absorbance of the resulting filtrate measured as described previously. Six standard curves, representing the three cultivation treatments imposed on thatch and soil were generated for each of 8 evaluation dates microbial activity was examined at the two sites. Standard curves were constructed by plotting absorbance as function of fluorescein concentration. Regression fits were

obtained using SAS (SAS Institute, 2001) PROC REG. Linear regressions obtained from the standard curves were used to calculate microbial activity, which was expressed in units of $\mu\text{g FDA hydrolyzed min}^{-1}\text{g}^{-1}$ media.

Moisture and organic matter content

The moisture content of cut bentgrass thatch and sieved soil and zoysiagrass thatch was determined by drying previously weighted material at 105 °C overnight. The percent moisture content of thatch or soil was calculated as the amount of moisture lost divided by sample dry weight.

Percent weight loss on ignition was used to determine the organic matter content of thatch and soil. The same thatch or soil samples that were used in the moisture content determination were used for organic matter determination. Both thatch and soil samples were dried at 60 °C for 48 h, weighed, and then combusted in a furnace at 550 °C for 3 hr (Blue M industrial oven, Model CFD-10E-7, New Columbia, PA). Percent organic matter was calculated as the difference in weight before and after sample combustion divided by dry weight of the sample.

Statistical Analysis

The field plot treatment structure used at both sites was a randomized complete block design with repeated measurements of microbial activity, moisture content, and organic matter content. Initial data analysis consisted of examining the strength of the linear relationships between microbial activity and media moisture content, and between microbial activity and media organic matter content at each site. The

coefficient of determination values for microbial activity and moisture content were generally superior to those obtained for organic matter content and microbial activity. Therefore moisture content was introduced as a covariate in the final analysis of the data. Data were analyzed using SAS PROC MIXED (SAS Institute, 2001). In the analysis microbial activity was the dependent variable and the examined factors included the three cultivation treatments, the two medium types and the three blocks. The four sampling dates at each site were the repeated time factor. Tukey's means separation analysis was used to compare the effect of cultivation treatment on thatch and soil microbial activity and thatch and soil organic matter content, for each of the four sample collection dates.

RESULTS

Thatch and Soil Microbial Activity

Creeping bentgrass site

Table 3.1 displays the general microbial activity of thatch and soil collected 15 to 58 days after imposing cultivation treatments on fairway managed creeping bentgrass. The general microbial activity of thatch ranged from 17.57 to 31.09 $\mu\text{g g}^{-1} \text{min}^{-1}$ (FDA hydrolyzed) over the course of the study while soil microbial activity ranged from 1.48 to 1.80 $\mu\text{g g}^{-1} \text{min}^{-1}$.

Creeping bentgrass thatch microbial activity 15 days after imposing the cultivation treatments was higher in hollow tine cultivated plots than in non-cultivated control plots. In contrast, thatch microbial activity was higher in control plots than in hollow tine cultivated plots 43 days after imposing the cultivation

treatments. No significant differences in thatch microbial activity were found between hollow tine cultivated plots and the control plots when samples were analyzed 28 and 58 days after treatments. Thatch microbial activity in vertically mowed turf was not significantly different from thatch microbial activity in the control plots on any of the four collection dates.

Soil microbial activity in hollow tine cultivated plots was significantly lower than that of the control plots 28 days after the imposition of the cultivation treatments. There were no significant differences in soil microbial activity between hollow tine cultivated plots and control plots on the other three field collection dates. Soil microbial activity in hollow tine cultivated plots was significantly higher than that of the vertically mowed plots 15 days after application. There was no difference in soil microbial activity between hollow tine cultivated and vertically mowed plots on any other collection dates. Soil microbial activity in vertically mowed turf was significantly lower than that of the control plots 28 days after application. No other significant differences in soil microbial activity between vertically mowed plots and control plots were found for the rest of the collection dates.

Zoysiagrass site

Table 3.2 displays the general microbial activity of thatch and soil collected 13 to 56 days after imposing cultivation treatments on the fairway zoysiagrass. The general microbial activity of thatch ranged from 14.63 to 20.78 $\mu\text{g g}^{-1} \text{min}^{-1}$ while the microbial activity of soil ranged from 1.86 to 2.24 $\mu\text{g g}^{-1} \text{min}^{-1}$.

Zoysiagrass thatch microbial activity in hollow tine cultivated plots was higher than that measured in the control plots 13 days after imposing the cultivation treatments. There were no significant differences in thatch microbial activity between hollow tine cultivated plots and control plots on any other date. Thatch microbial activity was lower in vertically mowed plots 40 days after cultivation, when compared to the control plots. No other significant differences in thatch microbial activity were found between vertically mowed plots and control plots for the rest of the collection dates.

Soil microbial activity in hollow tine cultivated plots was significantly higher than soil microbial activity in the control plots 40 and 56 days after application. There were no significant differences in soil microbial activity between hollow tine cultivated plots and control plots 13 and 27 days after cultivation. No significant differences in soil microbial activity between vertically mowed plots and control plots were found on any of the four collection dates.

Thatch and Soil Organic Matter Content

Creeping bentgrass site

The organic matter content in thatch and soil collected 15 to 58 days after imposing cultivation treatments on fairway managed creeping bentgrass is shown in Table 3.3. Thatch organic matter content, when averaged across cultivation treatments, was 620 and 641 g kg⁻¹ at the beginning and end of the study, respectively. There were no significant differences in thatch organic matter content between hollow tine cultivated plots and the control plots 15 and 28 days after

cultivation. Hollow tine cultivation significantly decreased thatch organic matter content 43 and 58 days after cultivation, when compared the non cultivated control treatment.

Soil organic matter content, when averaged across cultivation treatments, was 48 and 47 g kg⁻¹ at the beginning and end of the study, respectively. There was no significant difference in soil organic matter content between hollow tine cultivated plots and the control plots on any given date. Similarly, there was no significant difference in soil organic matter content between vertically mowed plots and the control on any given date.

Zoysiagrass site

The organic matter content in thatch and soil 13 to 56 days after imposing cultivation treatments at the zoysiagrass site is presented in Table 3.4. Thatch organic matter content, when averaged across cultivation treatments, was 552 and 519 g kg⁻¹ at the beginning and end of the study, respectively. Vertical mowing significantly decreased thatch organic matter content 13 days after imposing the cultivation treatments when compared to the non-cultivated control treatment. No significant difference in thatch organic matter content was found between the hollow tine cultivated plots and the control plots, or between vertical mowed plots and the control plots, for the rest of the collection dates.

Soil organic matter content when averaged across all cultivation treatments was 127 and 103 g kg⁻¹ at the beginning and end of the study, respectively. There was no significant difference in soil organic matter content between hollow tine cultivated

plots and the control plots on any given date. Similarly, there was no significant difference in soil organic matter content between vertically mowed plots and the control plots on any date.

Organic matter content, and moisture content regressions with microbial activity

The strength of the linear relationships between thatch and soil moisture content and microbial activity, and between thatch and soil organic matter content and microbial activity are shown in Table 3.5. The coefficient of determination (r^2) for the linear relationship between microbial activity and organic matter was 0.1299 for creeping bentgrass thatch and was 0.0311 for the soil at this site. Similarly, the r^2 for the linear relationship between microbial activity and organic matter at the zoysiagrass site was 0.1505 and 0.4939 for thatch and soil, respectively. The r^2 for the linear relationship between microbial activity and moisture content at the creeping bentgrass site was 0.9401 and 0.0662 for thatch and soil respectively. At the zoysiagrass site, the r^2 between microbial activity and media moisture content was 0.8964 for thatch and 0.4402 for soil. The slope for the linear relationships between microbial activity and thatch and soil moisture content are presented in Figures 3.1 and 3.2 for the creeping bentgrass and zoysiagrass sites, respectively. The slope of the linear relationship between microbial activity and moisture at the creeping bentgrass site was 0.0680 for thatch and 0.0104 for the soil. At the zoysiagrass site the slope of

the linear relationship between microbial activity and moisture was 0.0661 and 0.0110 for thatch and soil respectively.

DISCUSSION

In this study the level of general microbial activity was assessed by determining the rate of fluorescein diacetate (FDA) hydrolysis in thatch and soil. For both creeping bentgrass and zoysiagrass, thatch microbial activity was consistently higher than soil microbial activity. When averaged across cultivation treatments, creeping bentgrass thatch microbial activity was 15 times higher than microbial activity in the underlying soil. Similarly, there was 9 times more microbial activity in thatch than in soil when averaged across cultivation treatments at the zoysiagrass site. The results of this study are similar to those reported by Raturi et al. (2004) who found that creeping bentgrass and zoysiagrass thatch had an average total microbial biomass carbon amount that was 10 times greater than the microbial biomass carbon residing in the top 2 cm soil.

Elevated microbial activity in thatch has largely been attributed to the large pools of the decomposing plant material in thatch (Kerek et al., 2002). Bentgrass thatch microbial activity was 15 times higher than that of the underlying soil while thatch organic matter was 13 times higher than soil organic matter. In the case of zoysiagrass, thatch microbial activity was 9 times higher than soil microbial activity while thatch organic matter was 5 times higher than soil organic matter. These results support that a unit weight of thatch organic matter will support a higher level of microorganism activity than will a unit weight of soil organic matter. This is in

agreement with Raturi et al. (2004) who reported that the microbial biomass to total carbon ratio of zoysiagrass thatch was greater than the microbial biomass to total carbon ratio of the underlying soil. In their investigation Raturi et al. (2004) also measured the specific maintenance respiration rates in thatch and soil. Specific maintenance respiration rate per unit of microbial mass was lower in zoysiagrass thatch than the underlying soil. Raturi et al. (2004) proposed that the intense competition for the limited amount of organic carbon present in soil favors a microbe population in soil that requires higher amounts of carbon to survive than do microorganisms that reside in thatch.

On the first sampling date at both sites hollow tine cultivation increased thatch microbial activity when compared to the non cultivated control treatment. Thatch organic matter content in the hollow tine cultivated plots was not significantly different than thatch organic matter content in control plots at either site on the first sampling date. This would appear to indicate that hollow tine cultivation did not introduce sufficient soil into thatch to alter the moisture retention properties of thatch in a manner than would favor enhanced microbial activity. The measured moisture content of thatch in the hollow tine cultivated plots, however was higher than the measured moisture content of thatch collected from the non cultivated control plots at both site locations on the first sampling date (data not shown). The higher moisture content present in the thatch of the hollow tine cultivated plots likely enhanced thatch microbial activity within these plots compared to thatch in the control plots. No causal mechanism was identified that could account for the higher moisture levels seen in hollow tine cultivated plots. The same comparative moisture conditions

observed at the time of the first evaluation period between hollow tine cultivated and control plots were also observed on some later collection dates. However, the trends in moisture content seen on the other dates were not associated with an increase in thatch microbial activity within the hollow tine cultivated plots. This implies that while moisture has an important effect on thatch microbial activity, it is not the only factor influencing the microbial activity of thatch. Aeration status in the medium could be another factor that affected thatch microbial activity.

Hollow tine cultivation had little effect on thatch organic matter content. Thatch organic matter levels were consistently lower in hollow tine cultivated plots than the control plots at the zoysiagrass site, however none of the differences were statistically significant for the four evaluation dates. The only effect seen was a reduction in creeping bentgrass thatch organic matter content, when compared to the control plots, 58 days after imposing the cultivation treatments. These results indicate that changes in thatch organic matter content arising from hollow tine cultivation may not always occur immediately after cultivation. Murphy et al. (1993) suggested that the addition of soil to thatch with hollow tine cultivation may be responsible for lowering the fraction of organic matter in thatch, although no permanent reduction in total organic matter would result from cultivation. Danneberger and Turgeon (1986) found that making three passes over a thickly thatched Kentucky bluegrass lawn with a hollow tine cultivator increased the mineral content of the thatch from 18.5% to 66.1% when cores were returned and samples collected six weeks after cultivation. Danneberger and Turgeon (1986) collected no other measurements of thatch organic matter over the course of their investigation.

In the current study creeping bentgrass thatch organic matter content in the hollow tine treated plots was 12.5% lower than that measured in the thatch of the non cultivated control plots eight weeks after cultivation. The smaller effect of hollow tine cultivation on the incorporation of soil into thatch observed at the creeping bentgrass site compared to the amount reported by Danneberger and Turgeon (1986) may be due to grass species and soil differences at the two study site locations. When hollow tine cultivation was performed at the beginning of this study the soil texture at the creeping bentgrass site made it difficult to pulverize the soils cores pulled up by the cultivator. Nearly half of the soil cores remained on the surface of the hollow tine cultivated plots when the plots were mowed one week after imposing the cultivation treatments. These cores were removed when the plots were mowed. The removal of the large number of cores from the hollow tine cultivation plots at the creeping bentgrass site limited the amount of soil that was deposited back into the bentgrass canopy. The carpet like characteristics of the bentgrass canopy did not allow the cores to fall into the canopy, as may happen with turf that has a more vertical growth habit, and is mowed at a higher height of cut, than creeping bentgrass.

Hollow tine cultivation had no effect on soil organic matter content at the bentgrass or zoysiagrass site. One possible reason may be that intensity of the hollow tine cultivation treatment application made in this investigation was not sufficient to incorporate enough thatch organic matter into the soil to alter the organic matter content of the soil. If one assumes that all of the thatch removed by hollow tine cultivation is reincorporated into the underlying soil, the estimated maximum possible net gain in soil organic matter would be 1 g kg^{-1} at the bentgrass site and 2 g kg^{-1} at

the zoysiagrass site. These two estimations were based on the measured thickness and organic matter contents of the thatch at the two sites, the use of the creeping bentgrass and zoysiagrass thatch bulk density values by Raturi et al. (2004) (0.40 g cm^{-3} and 0.66 g cm^{-3} , respectively) and the portion of surface area from which cores were pulled (5% on area basis) in this investigation. It was also assumed that the soil bulk density at creeping bentgrass and zoysiagrass sites were 1.50 g cm^{-3} and 1.35 g cm^{-3} , respectively.

Vertical mowing had little effect on thatch and soil microbial activity. When compared to the non-cultivated control, vertical mowing reduced soil microbial activity at the creeping bentgrass site 28 days after treatment. Vertical mowing also reduced thatch microbial activity at zoysiagrass site 40 days after treatment. Organic matter data collected on aforementioned dates indicated that the lower level of microbial activity seen in the soil and thatch of the vertically mowed plots was not the result of changes in the organic matter content of the two media. In addition there were no differences in the thatch and soil moisture contents between the vertically mowed plots and the uncultivated control plots on the two dates of interest (date not shown). It is not clear why vertical mowing reduced microbial activity of the medium on the two aforementioned dates. This may warrant future investigations that would include qualitative measurements of thatch organic matter over time to better interpret the results obtained.

When linear regressions were performed between microbial activity and organic matter content, and microbial activity and moisture content, the r^2 for the relationship between thatch microbial activity and thatch moisture content was

superior to relationship between thatch microbial activity and thatch organic matter at both sites. Soil microbial activity was poorly correlated with soil moisture content and soil organic matter content at both sites. The relationship between microbial activity and soil moisture, and microbial activity and soil organic matter content, was slightly stronger at the zoysiagrass site than at the bentgrass site. Creeping bentgrass thatch microbial activity was ~ 6.5 times more sensitive to a unit change in thatch moisture content (slope=0.0680) than was soil microbial activity to unit change in soil moisture content (slope=0.0104). Similarly, zoysiagrass thatch microbial activity was more sensitive to changes in thatch moisture content (slope=0.0661) than was soil microbial activity to changes in soil moisture content (slope=0.0110).

The regression results indicate that thatch moisture content is a better predictor of thatch microbial activity than is thatch organic matter content. Linn and Doran (1984) noted that the percentage of soil pore space filled with water is closely related to soil microbial activity and that maximum aerobic microbial activity occurs at soil moisture levels between 50 and 70% of maximum water holding capacity of the soil. Soil moisture regulates oxygen diffusion in soil (Agehara and Warncke, 2005), which in turn affects aerobic microbial activity. While oxygen diffusion may control aerobic microbial activity in wet soil, substrate diffusion, on the other hand, has been hypothesized to be the main rate-limiting factor regulating aerobic microbial activity in dry soils (Schjønning et al., 2003). At low soil moisture levels declines in microbial activity may be due to limited diffusion of soluble substrates to microbes (Griffin, 1981; Agehara and Warncke, 2005). Killham et al. (1993) have suggested that declines in microbial activity in dry media may be a result of reduced microbial

mobility, which would also limit access to substrates. Thatch contains a much higher portion of macropores than soil (Hurto et al., 1980). The presence of a relatively few capillary pores in thatch causes this medium have to poor moisture retention properties which may limit microbial access to substrates in thatch. In the current study, creeping bentgrass thatch and soil moisture contents ranged from 113.12 to 410.88% (m/m) and from 17.16 to 28.41% (m/m), respectively. Zoysiagrass thatch and soil moisture contents ranged from 89.93 to 229.87% (m/m) and 32.35 to 66.75% (m/m), respectively. Thus thatch moisture content had a much larger range than that soil moisture content over the course of this investigation. This may partially explain why thatch microbial activity was much more sensitive to changes in moisture content than was soil microbial activity.

CONCLUSIONS

It is frequently stated that returning cores that are removed by hollow tine cultivation will improve the moisture retention properties of thatch. Such a moisture status improvement presumably provides a more favorable environment for microbial activity in thatch. In this investigation thatch and soil samples were collected at approximately 2 week intervals, for 56 to 58 days at two site locations. Thatch and soil were measured for general microbial activity, organic matter content and moisture content. Three passes of a hollow tine cultivator over creeping bentgrass and zoysiagrass turf with the cores returned did not consistently alter the organic matter content of either turf species thatch. Similarly, with the exception of one evaluation date, three passes of a vertical mower over creeping bentgrass and zoysiagrass turf

did not alter the thatch organic matter content of either turfgrass species when compared to turf that was not cultivated.

An examination of the strength of the relationship between microbial activity and thatch and soil moisture content and organic matter content revealed that microbial activity in these two media is more strongly correlated with, and sensitive to, changes in moisture content than with changes in organic matter content. When compared to non-cultivated control plots a consistent enhancement in thatch microbial activity was observed 13 to 15 days after performing hollow tine cultivation. The enhancement in activity was associated with higher moisture levels within the thatch samples collected from the hollow tine cultivated plots compared to non-cultivated plots. Hollow tine cultivation had no consistent effect on soil microbial activity or on thatch microbial activity beyond that observed on the initial sample collection date. When compared to uncultivated control plots, vertical mowing had no consistent effect on thatch or soil microbial activity at either site.

While hollow tine cultivation and vertical mowing are important and necessary management practices used to improve soil physical properties and restore turf vitality, the results of this study do not extend the benefits associated with these two cultivation practices to include an extended enhancement in microbial activity. The results of this study also suggest that irrigation management will have a larger influence on microbial activity in turf than will thatch management.

Table 3.1. General microbial activity of thatch and soil collected 15 to 58 days after imposing cultivation treatments on fairway managed creeping bentgrass located at the Paint Branch Turfgrass Research Facility in College Park, Maryland.

Media	Cultivation [†]	Day after cultivation			
		15	28	43	58
		————— μg Fluo./ g (media) / min —————			
Thatch	Vertical mow (VM)	22.87 ab‡	30.70 a	23.32 ab	17.57 a
	Hollow tine (HT)	24.68 a	31.09 a	21.82 b	18.50 a
	None (N)	21.93 b	30.71 a	24.51 a	18.67 a
Soil	Vertical mow (VM)	1.65 B	1.58 B	1.62 A	1.54 A
	Hollow tine (HT)	1.80 A	1.52 B	1.56 A	1.51 A
	None (N)	1.71 AB	1.74 A	1.62 A	1.48 A

[†] Field cultivation treatments included three vertical mower passes over a plot with the debris created by the treatment removed (VM), three passes over the plot with hollow tine cultivator with the cores returned (HT), and a non-cultivated control (N). All field treatments were imposed once on April 16, 2008.

‡ Values followed by the same case letter within a column are not significantly different from one another at the 0.05 level by Tukey's mean separation test. Means followed by upper case letters were analyzed separately from means followed by lower case letters, thus means followed different case letters should not be compared with one another.

Table 3.2. General microbial activity of thatch and soil collected 13 to 56 days after imposing cultivation treatments on a zoysiagrass fairway located at the Woodmont Country Club in Rockville, Maryland.

Media	Cultivation [†]	Day after cultivation			
		13	27	40	56
		————— μg Fluo./ g (media) / min —————			
Thatch	Vertical mow (VM)	14.63 b‡	19.29 a	18.07 b	20.46 a
	Hollow tine (HT)	15.03 a	18.65 a	20.28 ab	19.26 a
	None (N)	14.65 b	18.44 a	20.78 a	21.02 a
Soil	Vertical mow (VM)	2.24 A	2.19 A	1.93 AB	2.02 AB
	Hollow tine (HT)	2.16 A	2.15 A	2.05 A	2.10 A
	None (N)	2.13 A	2.24 A	1.86 B	1.90 B

[†] Field cultivation treatments included three vertical mower passes over a plot with the debris created by the treatment removed (VM), three passes over the plot with hollow tine cultivator with the cores returned (HT), and a non-cultivated control (N). All field treatments were imposed once on May 27, 2008.

‡ Values followed by the same case letter within a column are not significantly different from one another at the 0.05 level by Tukey's mean separation test. Means followed by upper case letters were analyzed separately from means followed by lower case letters, thus means followed different case letters should not be compared with one another.

Table 3.3. Organic matter content of thatch and soil collected 15 to 58 days after imposing cultivation treatments on fairway managed creeping bentgrass located at the Paint Branch Turfgrass Research Facility in College Park, Maryland.

Media	Cultivation [†]	Day after cultivation			
		15	28	43	58
		g kg ⁻¹			
Thatch	Vertical mow (VM)	664.89 a‡	659.67 a	638.78 a	644.78 ab
	Hollow tine (HT)	618.67 ab	646.56 a	552.89 b	597.00 b
	None (N)	576.89 b	649.22 a	590.67 ab	681.89 a
Soil	Vertical mow (VM)	47.56 A	47.89 A	46.56 A	45.89 A
	Hollow tine (HT)	47.11 A	46.89 A	49.44 A	45.44 A
	None (N)	48.44 A	47.33 A	48.78 A	48.11 A

[†] Field cultivation treatments included three vertical mower passes over a plot with the debris created by the treatment removed (VM), three passes over the plot with hollow tine cultivator with the cores returned (HT), and a non-cultivated control (N). All field treatments were imposed once on April 16, 2008.

‡ Values followed by the same case letter within a column are not significantly different from one another at the 0.05 level by Tukey's mean separation test. Means followed by upper case letters were analyzed separately from means followed by lower case letters, thus means followed different case letters should not be compared with one another.

Table 3.4. Organic matter content of thatch and soil collected 13 to 56 days after imposing cultivation treatments on a zoysiagrass fairway located at the Woodmont Country Club in Rockville, Maryland.

Media	Cultivation [†]	Day after cultivation			
		13	27	40	56
		g kg ⁻¹			
Thatch	Vertical mow (VM)	477.22 b‡	511.56 a	614.44 a	545.00 a
	Hollow tine (HT)	559.56 ab	529.44 a	506.11 a	493.67 a
	None (N)	620.11 a	580.78 a	547.33 a	519.00 a
Soil	Vertical mow (VM)	123.11 A	104.44 A	101.44 A	101.33 A
	Hollow tine (HT)	135.56 A	103.89 A	109.33 A	95.89 A
	None (N)	123.00 A	112.78 A	110.89 A	112.89 A

[†] Field cultivation treatments included three vertical mower passes over a plot with the debris created by the treatment removed (VM), three passes over the plot with hollow tine cultivator with the cores returned (HT), and a non-cultivated control (N). All field treatments were imposed once on May 27, 2008.

[‡] Values followed by the same case letter within a column are not significantly different from one another at the 0.05 level by Tukey's mean separation test. Means followed by upper case letters were analyzed separately from means followed by lower case letters, thus means followed different case letters should not be compared with one another.

Table 3.5. Coefficients of determination for linear regressions between microbial activity and organic matter content, and between microbial activity and moisture content for thatch and soil at both sites.

Regression	Medium	Coefficient of determination	
		Creeping bentgrass	Zoysiagrass
Microbial activity vs. Organic matter	Thatch	0.1299	0.1505
	Soil	0.0311	0.4939
Microbial activity vs. Moisture	Thatch	0.9401	0.8964
	Soil	0.0662	0.4402

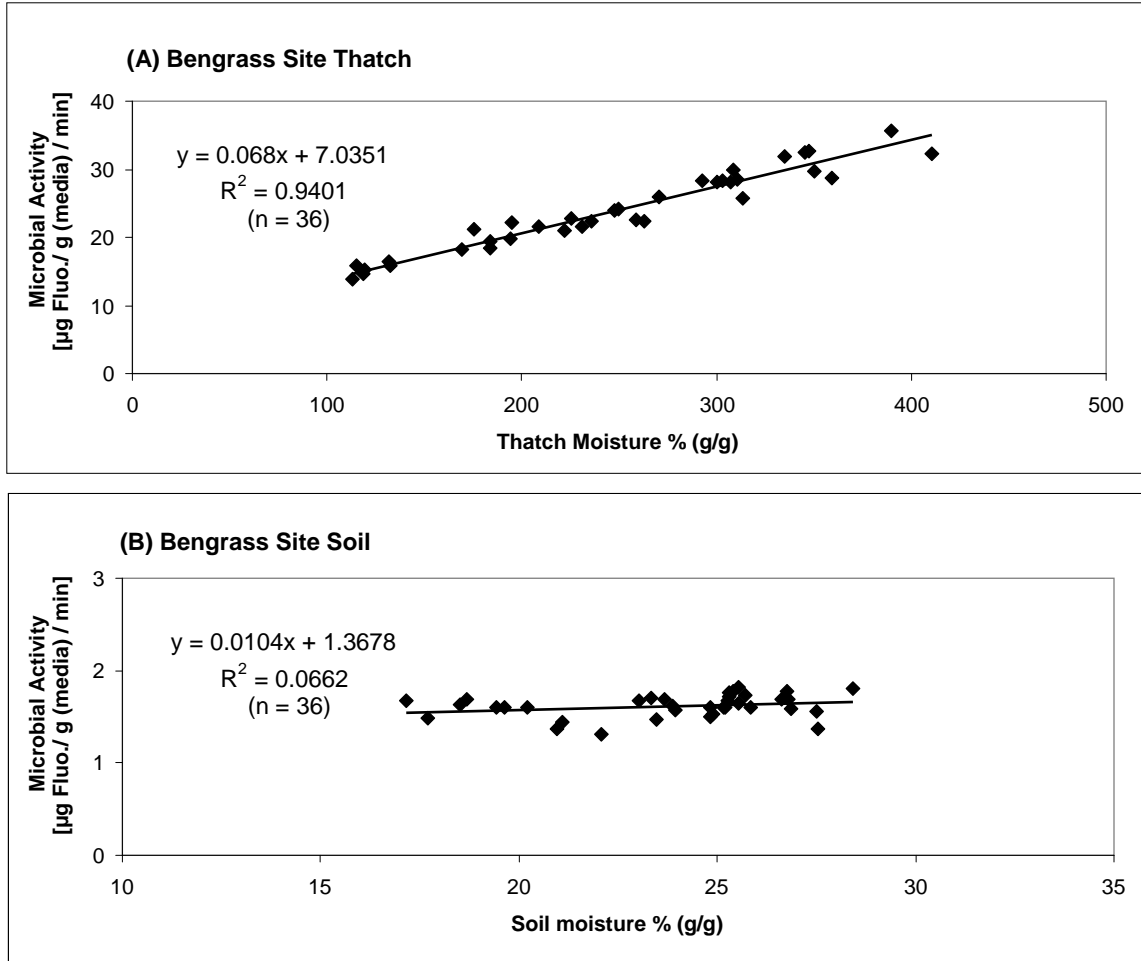


Figure 3.1. The effect of thatch moisture content (A), and the moisture content in the top 2.5 cm of soil (B), on the total microbial activity in each medium at the creeping bentgrass site in College Park, MD.

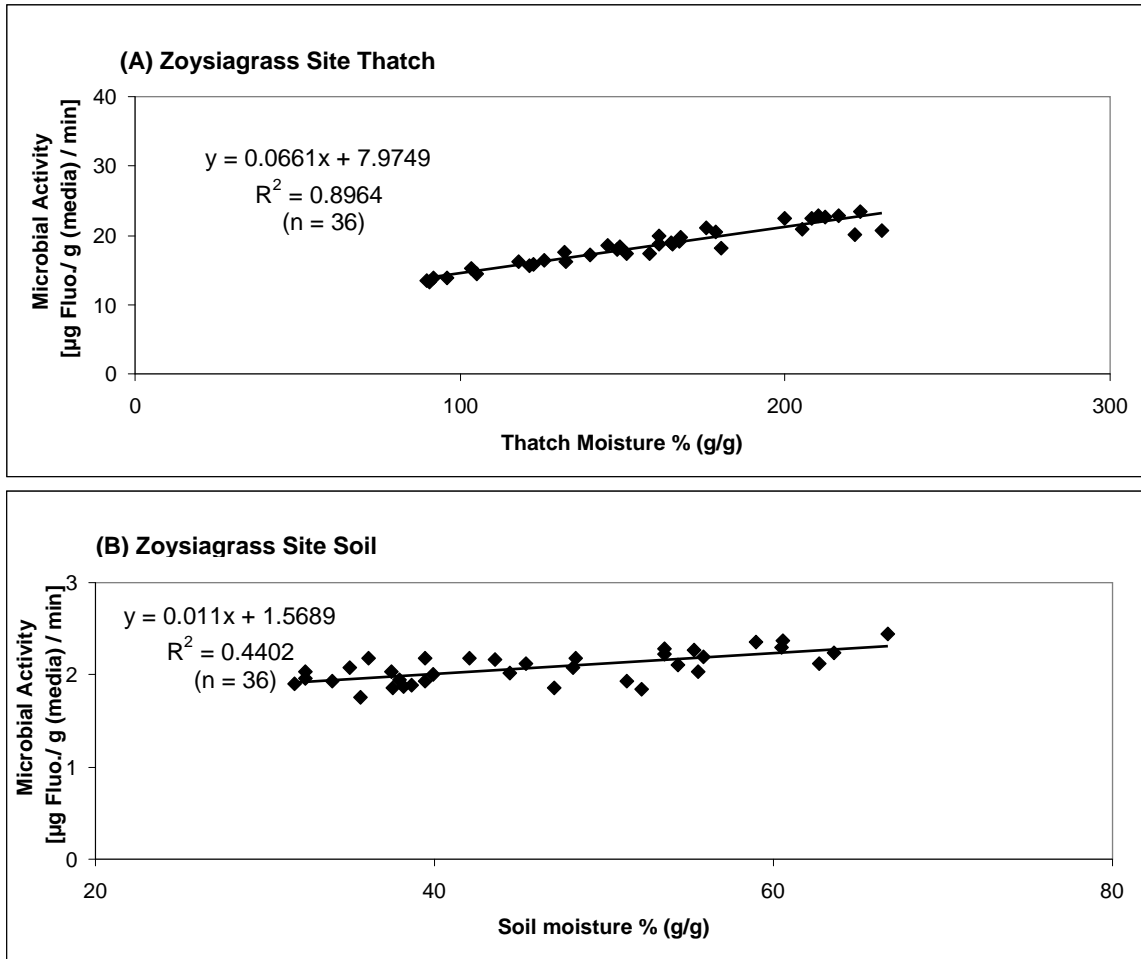


Figure 3.2. The effect of thatch moisture content (A), and the moisture content in the top 2.5 cm of soil (B), on the total microbial activity in each medium at the Woodmont Country Club zoysiagrass site in Rockville, MD.

CHAPTER IV.

DEGRADATION OF PESTICIDES HAVING DIFFERING SORPTION AFFINITIES IN THATCH AND SOIL SUBJECTED TO VERTICAL MOWING AND HOLLOW TINE CULTIVATION

Turfgrass is the predominant feature found in urban and suburban landscapes. One indispensable tool in managing turf is the use of pesticides. When used selectively, herbicides, fungicides, and insecticides are beneficial tools that will help ensure acceptable turf density is maintained. This can be particularly important for golf courses, where the use of pesticides not only is considered for aesthetics purposes, but required in order to maintain the functional performance of fine golf turf.

Degradation is one of the primary processes responsible for dissipation of pesticides in porous media. Degradation pathways include microbial degradation, chemical degradation and photodegradation (Wheeler, 2002). Microbial degradation is the transformation of the pesticide by fungi, bacteria and other microorganisms that can use it as an energy source. Aerobic microbial degradation is the primary means by which many pesticides are lost from porous media (Pothuluri et al., 1990; Sylvia et al., 1997).

In mineral soils it is generally accepted that the microbial degradation of a pesticide is inhibited when a pesticide partitions into the macromolecules of organic matter, or the small spores and fissures in organic matter aggregates (Beulke et al.,

2005). Thatch is an enriched organic medium that resides between the soil surface and foliar layer of a turfgrass canopy. The high organic matter content of this medium readily sorbs water insoluble pesticides. This medium also supports a higher level of microbial activity than most mineral soils (Mancino et al., 1993; Raturi et al., 2004). Several field studies have observed that pesticide dissipation in thatch is generally quicker than in soil (Horst et al., 1996; Gardner et al., 2000; Gardner and Branham, 2001).

Pesticide aerobic degradation is typically measured in batch incubation experiments (Gaston et al., 2003). Few batch incubation experiments have investigated the degradation of pesticides in thatch. The few pesticide incubation studies that have examined the degradation of pesticides in thatch and soil suggest that enhanced pesticide degradation in thatch is limited to pesticides having low to moderate sorption affinities. Roy et al. (2001) examined the degradation of minimally sorbed dicamba (3,6-dichloro-2-methoxybenzoic acid) ($K_{oc} = 1$) to thatch and soil and reported dicamba degradation was 5.9 to 8.4 times greater in thatch than in the soil. Similarly, Wu et al. (2002a) examined the degradation of minimally sorbed metalaxyl [*N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl)alanine methyl ester] ($K_{oc} = 50$) in thatch and soil and reported that the half life of metalaxyl in thatch was 110 days, compared to 165 days in soil. In another study Wu et al. (2002b) documented that the degradation of strongly adsorbed chlorpyrifos (*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridylphosphorothioate) ($K_{oc} = 6070$) was slower in thatch (half life = 248 days) than in soil (half life = 120 days). To date only a single study appears to have examined the degradation of turf-applied pesticides having contrasting sorption

properties in thatch and soil (Frederick et al., 1994). Frederick et al. (1994) examined degradation of three pesticides with Koc ranging from 100 to 1650 (i.e., vinclozolin, triadimefon, and chloroneb) and found that degradation of all three pesticides was slower in thatch than in soil.

Two commonly used cultivation practices in turf management are hollow tine cultivation and vertical mowing. The hollow tines of a cultivator can pull soil cores up while the vertically rotating blades of a vertical mower can slice into thatch and bring plant debris up from below the turf canopy. It is often stated that these two cultivation practices have the potential to improve biological conditions within thatch (Hurto et al., 1980; Berndt, 2008). One such claim, for example, is that when soil cores removed by hollow tine cultivation are incorporated into the turf, microbial activity within thatch will improve (Hurto et al., 1980). It is not well understood, however, how such a potential enhancement in microbial activity may affect pesticide microbial degradation in turf.

Given the important role thatch has in retaining pesticides, a better understanding of the interaction that exists between pesticide sorption and pesticide degradation within thatch is needed. This is especially true in light of the fact that only a single incubation study appears to exist in the literature that has examined the degradation of pesticides having contrasting sorption properties (Frederick et al., 1994). In current turf pesticide fate modeling efforts the pesticide persistence parameter used in a model is frequently obtained from aerobic soil half life values listed in United States Department of Agriculture, Agriculture Research Service (USDA-ARS) pesticide properties data base (Haith, 2001; Haith et al., 2002). The

USDA-ARS pesticide dissipation data base was developed by relying almost exclusively on investigations involving mineral soils. A more complete understanding of pesticide microbial degradation in thatch would aid modelers in deciding how to adjust the USDA-ARS pesticide dissipation degradation constants to better represent how thatch influences the persistence of pesticides that are applied to turf.

The objectives in this study were to 1) determine how the sorption properties of a pesticide may alter the relative aerobic half lives of a pesticide in thatch and soil, and 2) to examine the effect of hollow tine cultivation and vertical mowing on pesticide degradation. The working hypothesis of this investigation is that minimal sorbed pesticides should degrade faster in thatch than in soil while readily sorbed pesticides may degrade more slowly in thatch than in soil. The objectives were evaluated by comparing the degradation of three pesticides having widely different sorptive properties. The degradation of the three pesticides in thatch and soil were examined using traditional batch incubation techniques.

MATERIALS AND METHODS

Site Description

The thatch and soil used in this investigation were collected from a large block of 'L-93' creeping bentgrass that was located at the University of Maryland Paint Branch Turfgrass Research Facility in College Park, MD. The turf was established from seed in May of 2004. The soil at the site was mapped as Keyport silt loam (fine, mixed, semiactive, mesic Aquic Hapludult). The surface 2.5 cm of soil contained 34.3% sand, 46.6% silt, 19.1% clay, 48 g kg⁻¹ organic matter, and had a pH of 5.2.

The thatch at this site contained 458 g kg⁻¹ organic matter and had a pH of 5.9. Visual inspection of the thatch revealed it consisted of a tightly matted network of live and partially decomposed fine textured stolons intermingled with a mass of white and tan colored roots. Prior to this investigation the block was maintained as a simulated golf turf fairway for the purpose of conducting nutrient and pesticide runoff investigations. In the year preceding this investigation the turf received a single tank mix application of 44 g ha⁻¹ dicamba (3,6-dichloro-2-methoxybenzoic acid), 81 g ha⁻¹ MCPP (methylchlorophenoxypropionic acid), 17 g ha⁻¹ 2,4-D, 1.11 kg ha⁻¹ flutolanil and 155 g ha⁻¹ chlorpyrifos on 23 July 2007. The most recent cultivation practice performed on the site prior to the initiation of this investigation was two passes of a vertical mower on 28 Mar. 2007 with the blades of the vertical mower set to penetrate the surface 0.25 cm of soil.

Three cultivation treatments were imposed on 16 April 2008 immediately after mowing the turf. The cultivation treatments were imposed on 3.0 m x 3.0 m plots with each treatment being replicated three times utilizing a randomized complete block design. The three cultivation treatments were no cultivation (N), three passes over the plot with hollow tine cultivator with the cores returned (HT), and three vertical mower passes over the plot with the debris created by the treatment removed (VM). The HT cultivation treatment was imposed using a Jacobsen Lawnaire IV aerator (Jacobsen, a Textron Company, Charlotte, NC) equipped with 1.9 cm diameter tines. The aerator created a 9.5 cm x 18 cm hole pattern and was set to extract soil cores to a depth of 7 cm. A Bluebird EasyScape lawn comb, (BlueBird Lawn Comber Company, Beatrice, NE) with a 5 cm spacing between blades, was

used for vertical mowing, with the depth of penetration set to 4.0 cm. The three passes within each plot were executed in such a way that two of the passes were made along the two perpendicular sides of the square and the third pass was made along the direction of the diagonal. Debris created by vertical mowing was removed from the plot on 16 April with a leaf rake. On 17 and 18 April, a metal rake was used to break up soil cores left on the turf canopy of the hollow tine cultivated plots. Also on 18 April, the plot area received 48.8 kg N ha⁻¹, as urea (46-0-0), with 0.6 cm of water being applied to the plot area to wash the urea into the underlying soil. Prior to and during this investigation the turf was mowed 2 to 3 times week at a bench height of 1.25 cm. Mowing of the plot area was briefly suspended after imposing the cultivation treatments, but was reinitiated on 23 April 2008. Soil remaining on the turf surface of the hollow tine cultivated plots was removed on 23 April 2008 when plots were mowed.

Sample processing and incubation procedure

On 6 July 2008 a Ryan Jr. model 544844 sod cutter (Ryan, Div. of Schiller Grounds Care, Inc., Johnson Creek, WI) was used to collect thatch and soil from each of the 9 plot that received one of the three cultivation treatments. The sod cutter was set to a depth of cut of 4 cm to ensure 2.5 cm of soil was collected. The sod strips were transported to the lab where small portions of the sod strips were cut out using a hack saw blade. The verdure was discarded and thatch and soil were then separated using the same hack saw blade, which was rinsed with ethyl alcohol and air dried between processing thatch and soil collected from different plots. The thatch was

passed through a 4 mm sieve, and soil through a 2 mm sieve, to homogenize each medium. Half of the sieved thatch and soil from each cultivation treatment were autoclaved at 121 °C for 55 min. three separate times. Samples that were autoclaved were subjected to this procedure at 2-day intervals. This resulted in media sterilization being completed in 6 days.

The incubation procedure consisted of placing 1 g dry weight equivalent of thatch, or 10.0 g dry weight equivalent of soil, into a 50 ml centrifugeable polypropylene sterile incubation tube. This was done for autoclaved and non-autoclaved thatch and soil collected from each field plot. Sample materials were not packed after being placed in the tubes. The respective media were then brought to field capacity by adding an appropriate amount of autoclaved water to each incubation tube. The field capacity moisture content of thatch and soil were determined in the previously mentioned nutrient and pesticide runoff investigation in this thesis. In the earlier runoff investigation intact thatch and soil cores that were extracted from the field site were saturated, placed on a ceramic plate having bubbling pressure of 100 kpa, and subjected to pressure of 33 kpa while within a vacuum pressure extractor. The mass basis field capacity moisture content (i.e. 33 kpa) for soil and thatch were found to be 0.191 and 1.58, respectively. After samples had been brought to field capacity, 1 mL of a 100% methanol solution that contained 18 mg L⁻¹ 2,4-D, 533 mg L⁻¹ flutolanil, and 73 mg L⁻¹ chlorpyrifos was added to each incubation tube. On a surface area basis the 1 mL addition of the pesticide containing solution was equivalent to making a field rate application of 0.27 kg ha⁻¹ 2,4-D, 8 kg ha⁻¹ flutolanil, and 1.1 kg ha⁻¹ chlorpyrifos. These application rates were equivalent to

the maximum label rate for each pesticide if applied to bentgrass in 2007. Following the addition of the methanol a sterile cotton plug was inserted into the top of each incubation tube. The tubes were then placed in an incubator (American Scientific Products Incubator, model IC-62, Columbus, OH) maintained at 25°C. At approximately 4 day intervals for the duration of the study, individual tubes were weighted and the amount of autoclaved deionized water needed to return the medium field capacity added to the tube. At times of 2 hr, 1 day, 2 days, 4 days, 8 days, 24 days, 48 days and 96 days after addition of pesticide containing solution, select incubation tubes were removed from the incubator and placed into a freezer maintained at -20 °C to stop pesticide microbial degradation within the medium.

Pesticide extraction

Two individual thatch or soil samples were required to extract all three pesticides. One procedure was used to extract 2,4-D from the thatch or soil present in one tube while another procedure was used to extract flutolanil and chlorpyrifos from the same medium present in a second incubation tube. The extraction of 2,4-D closely followed the method used by Gan et al. (2003). In this method 15 mL of a 1:1 methanol-water (v/v) solution was added to the thatch or soil after it was allowed to warm to room temperature. The incubation tubes containing the respective medium were then tightly capped and the tubes shaken on an end-to-end shaker for 2 hr, after which the tubes were centrifuged at 700 x g (relative centrifugal force) for 10 min. An aliquot of the supernatant was then passed through a 0.2 µm filter and resulting filtrate placed into a 2 mL autosampler vial. The procedure used to extract flutolanil

and chlorpyrifos was similar to that used for 2,4-D except that 15 mL of iso-octane (2,2,4-trimethylpentane) was used in place of the 1:1 methanol-water solution to extract these two pesticides. In addition, the aliquot of supernatant obtained after centrifuging a sample was not passed through a filter, but was directly transferred to a 2 mL autosampler vial. For all three pesticides, solutions placed into the autosampler vials were either stored at -20 °C or were analyzed immediately as described in the following section.

Pesticide residue analysis

The concentration of 2,4-D present in the samples that were extracted using a 1:1 methanol-water extract were determined by High Performance Liquid Chromatography. The analysis was performed using Hewlett Packard 1100 Series Liquid Chromatography Station that was equipped with a diode array detector and a Zorbax Eclipse XDB-C8 column (150 mm long x 4.6-mm i.d., 5- μ m particle size). The mobile phase solvent consisted of a 55% acetonitrile and 45% acidified water (by volume) solution that was passed through the system at a flow rate of 1 ml min⁻¹. The acidified water was a 2.5% acetic acid solution (by volume). The injection volume was 500 μ L and detection was by UV absorbance at 230 nm, with a retention time of 4.2 min for 2,4-D. Average method recoveries for 2,4-D in thatch and soil were 86% and 70%, respectively.

The concentration of flutolanil and chlorpyrifos present in the iso-octane extracted samples were determined by Gas Chromatography using a Hewlett Packard 5890 Series II gas chromatograph. A 2.0- μ L aliquot of sample was injected into a

J&W capillary column (30 m, 0.53 mm i.d. x 1.0 μm thickness) using a nitrogen carrier gas pressure of 3.5 psi and a 3 mL min^{-1} flow rate. The inlet temperature was 200 $^{\circ}\text{C}$ and the ECD temperature was 300 $^{\circ}\text{C}$. A temperature gradient was used, starting at 160 $^{\circ}\text{C}$ for 3 min, and then increasing to 300 $^{\circ}\text{C}$ at 30 $^{\circ}\text{C min}^{-1}$. The final temperature was held for 5 min. Retention times were 7.2 min for chlorpyrifos and 7.8 min for flutolanil. Average method recoveries for thatch and soil were 108.8% and 94.3% for chlorpyrifos and 97.5% and 84.2% for flutolanil, respectively.

Statistical Analysis

Pesticide degradation data obtained from both the HPLC and GC analyses were conventionally analyzed by assuming pesticide degradation followed first-order kinetics: $C_t = C_0 e^{-kt}$, where C_t is the amount of pesticide at time t after application, C_0 is the amount of pesticide present in the turf immediately after application, and k is the degradation rate constant of the pesticide. The pesticide concentration data (C_t) were transformed to the natural log values to convert the relationship between C and t to linear form. Analysis of covariance with unequal variances between treatments was used to estimate and compare the linear slope k between the specific treatment combinations of interest. Analysis was carried out in PROC MIXED with ESTIMATE statements being used to obtain the k values of interest and their matching standard errors (SAS Institute, 2001). The time for the dissipation of 50% of the initial amount (half-time, $t_{1/2}$) of the pesticides was calculated using k and the following relationship; $t_{1/2}$ (day) = $\ln(2)/k$ (day^{-1}).

RESULTS

First order degradation constants, and statistics summarizing the measure of confidence associated with the degradation constants for the 3-way interaction of medium, autoclaving and cultivation on 2,4-D, flutolanil and chlorpyrifos degradation are presented in Tables 4.1- 4.3. The coefficient of variation (CV) for the 3 way interaction degradation constants ranged from 5.7 to 29.4% for 2,4-D (Table 4.1), 6.9 to 33.1% for flutolanil (Table 4.2) and from 9 to 31.5% for chlorpyrifos (Table 4.3). Degradation constants for the 2-way interaction of medium and autoclaving on 2,4-D, flutolanil and chlorpyrifos degradation are presented in Table 4.4 along with the standard error and CV of each constant. The CV for the medium by autoclaved soil degradation constant was higher than the CV for the non-autoclaved soil, non-autoclaved thatch and autoclaved thatch degradation constants for all three of the pesticides that were examined. In the case of 2,4-D and flutolanil, there was a greater amount of uncertainty associated with the non-autoclaved thatch degradation constants than with the non-autoclaved soil degradation constants. In a prior incubation investigation involving thatch and soil, Frederick et al. (1994) reported that the CV in the two media range from 12.5 to 15.4% for chloroneb, 12.5 to 25.0% for triadimefon, and 11.1 to 31.4% for vinclozolin.

Significance probabilities for the degradation constant comparisons of interest are presented in Tables 4.5, 4.6 and 4.7 for 2,4-D, flutolanil and chlorpyrifos, respectively. Cultivation had no effect on 2,4-D or chlorpyrifos degradation in thatch or soil. The effect of cultivation on flutolanil degradation was limited to autoclaved soil. Hollow tine cultivation performed 80 days prior to sample collection

significantly reduced ($P = 0.0382$) flutolanil degradation when compared to soil that was not subjected to cultivation. The incubation degradation half life in the autoclaved hollow tine cultivated soil was 103.5 days, compared to 87.9 days in the autoclaved non-cultivated soil.

Autoclaving thatch and soil slowed 2,4-D degradation in soil, but had no effect on 2,4-D degradation in thatch (Table 4.5). In non-autoclaved thatch and soil 2,4-D degradation was slower in thatch than in soil. In thatch and soil that were autoclaved, 2,4-D degradation was faster in thatch than in soil. The half life of 2,4-D in autoclaved thatch and soil were 48 and 69.2 days, respectively (Fig 4.1). In non-autoclaved thatch and soil the half life of 2,4-D was 45.3 and 25.7 days, respectively.

Autoclaving significantly slowed flutolanil ($P = 0.0268$) and chlorpyrifos ($P = 0.0366$) degradation in thatch, but had no effect on the degradation of these two pesticides in soil (Table 4.6). When pooled over the three cultivation treatments the half life of flutolanil in autoclaved thatch was 126 days, compared to a half life of 87.7 days in non-autoclaved thatch (Fig 4.1). Similarly, the degradation half life for chlorpyrifos in autoclaved thatch was 82.1 days, compared to a half life of 67.3 days in thatch that was not autoclaved. The degradation of flutolanil and chlorpyrifos in non-autoclaved thatch were no different than the degradation of flutolanil and chlorpyrifos in non-autoclaved soil (Fig 4.2). Similarly flutolanil and chlorpyrifos degradation in autoclaved thatch were no different than that measured in autoclaved soil.

DISCUSSION

The effect of microbial activity on pesticide degradation within a medium can be evaluated by comparing the degradation constants for autoclaved and non-autoclaved media (Roy et al., 2001). Degradation constants (and their associated half life) observed in autoclaved media are assumed to be due to abiotic transformations only. Degradation constants obtained in non-autoclaved media are assumed to be due to both biotic and abiotic transformations. An inherent assumption that is made when the degradation constants for autoclaved and non-autoclave media are compared is that the autoclaved medium remains sterile for the duration of the pesticide degradation evaluation period.

In this investigation the degradation constants for flutolanil and chlorpyrifos were higher in non-autoclaved thatch than in autoclaved thatch. This indicates that microbial activity is an important process in the degradation of these two pesticides within thatch. In contrast, there was little difference in the flutolanil and chlorpyrifos degradation constants between autoclaved and non-autoclaved soil. This indicates that for the conditions of this investigation, microbial activity was not an important avenue of flutolanil and chlorpyrifos degradation in soil.

The apparent extent of chlorpyrifos degradation attributable to microbial activity seen in thatch was smaller than reported in other high organic matter content media. Miles et al. (1984) examined the degradation of chlorpyrifos in peat that had an organic matter content comparable (i.e., 577 g kg⁻¹ vs. 458 g kg⁻¹) to the thatch examined in this investigation. The highest moisture content maintained in the incubation investigations of Miles et al. (1984) was 60% of the available moisture

holding capacity (AMC) of peat. This was somewhat drier than the moisture content (i.e., field capacity) at which thatch was maintained in the current investigation. The half life for chlorpyrifos in sterile peat at 60% AMC was ca. 126 days compared to a half life of 7 to 42 days in non-sterile peat. The half life values presented by Miles et al. (1984) indicate that sterilization of peat resulted in at least a 200% increase in the half of life of chlorpyrifos, when compared to half life obtained in non-sterile peat. In contrast, sterilization of thatch by autoclaving resulted in a 22% increase in the half of life of chlorpyrifos, when compared to the half life obtained in non-sterile thatch.

The small differences seen in the degradation of chlorpyrifos in autoclaved and non-autoclaved soil may be related to the fact that chlorpyrifos degradation is often times dominated by abiotic processes. Racke et al. (1996) examined chlorpyrifos degradation in sterile and non-sterile soils. In their study 37 different soils with pH values ranging from 3.8 to 8.5 were examined and soil sterilization was achieved by subjecting soil to γ -irradiation. They found that microbial and hydrolytic mechanisms both accounted for chlorpyrifos degradation in soil. Chlorpyrifos hydrolysis however was greatly accelerated in both acidic and alkaline soils under low moisture conditions. In the current study the addition of water to the incubation vessels every 4 to 6 days insured that moisture content of soil was at or slightly below field capacity throughout the entire incubation period. The degradation constants obtained in the current investigation nevertheless suggest that there were few chlorpyrifos degrading organisms present within soil, and that almost all of the chlorpyrifos degradation that occurred was the result of abiotic transformations.

No study appears to exist in the literature that has examined the degradation of

flutolanil in high organic content media. A previous investigation that examined the degradation of flutolanil in mineral soil however tends to support the soil results obtained in this investigation. Suzuki et al. (2001) examined the degradation of flutolanil in soil that was placed into open containers, or was placed into the same containers that were then sealed to maintain anaerobic soil conditions for the duration of the incubation experiment. The half life of flutolanil in the well aerated soil was 336 days compared a half life of 361 days in the anaerobic soil environment.

Although the results of Suzuki et al (2001) were not based on autoclaved and non-autoclaved soils, the similarity in the aerobic and anaerobic half lives suggests that aerobic microbial activity is not the primary process responsible for the degradation of flutolanil in soil. The flutolanil degradation half life in non-autoclaved soil measured in the current investigation was similar to that reported for two agricultural soils by Suzuki and Otani (2004). In the investigation conducted by Suzuki and Otani (2004) the half of flutolanil in a loam and clay loam soil were 82.5 and 76.4 days respectively, compared to a half life of 75.3 day in the non-autoclaved soil of this study.

When compared to non-autoclaved thatch and soil, autoclaving had no effect on 2,4-D degradation in thatch. Autoclaving soil however significantly slowed 2,4-D degradation in this medium. The thatch results are at odds with numerous investigations that have reported many microorganisms are capable of metabolizing 2,4-D (Sandmann et al., 1988). One possible explanation for the differing thatch and soil 2,4-D results may be that the composition of the microbial population in thatch was different than that in soil. The 2,4-D-degrading organisms residing in thatch may

have been more sensitive to the methanol solution that was used to add pesticides to each media than were 2,4-D degrading organisms present in soil. The potentially deleterious effect of methanol on 2,4-D-degrading organisms has been mentioned by Guo et al. (2000) who avoided this concern by limiting the proportion of incubated medium that received methanol to 2.5% (m/m). The small portion of soil that was fortified with the 2,4-D containing methanol solution was remixed with a larger mass of soil material 24 hours after the initial methanol addition had been made. The resulting composite sample was then incubated for the time period required. In the current investigation thatch and soil were brought to field capacity prior to adding the pesticide containing methanol solution. Visually it appeared that the 1-mL addition of the pesticide containing methanol solution made to thatch and soil was sufficient to result in near complete fortification of both media. Given that the mass of an individual soil sample was 10 times that of thatch sample, it is possible that fortification may have been more complete in thatch than in soil. This would account for the differential response seen in the two media without regard to population differences in 2,4-D degrading bacteria that may exist in thatch and soil. The USDA-ARS pesticide properties database lists the aerobic soil half life of 2,4-D as 5.5 days. The 2,4-D half life for the non-autoclaved soil in this investigation was 25.7 days. The longer 2,4-D aerobic soil half life seen in this investigation provides additional evidence that the methanol solution used to add the three pesticides examined in the investigation may have been toxic to 2,4-D-degrading organisms.

Cultivation had minimal effect on pesticide degradation in this study. Only one of the 24 cultivation treatment comparisons examined (i.e., 8 for each pesticide) was

found to be significant. The flutolanil degradation rate constant for the non-autoclaved soil hollow tine treatment was significantly larger (i.e., shorter half life) than that flutolanil degradation rate constant for the non-autoclaved control soil treatment. Given that the role of aerobic microbial degradation appears to be of minor importance in the degradation of flutolanil, it is unlikely that an improvement in the aeration properties of the soil brought about by hollow tine cultivation would explain the accelerated rate of decay of flutolanil seen in hollow tine treated soil. Moreover, because all soil was passed through a 2 mm sieve prior to placing this media in the incubation vessels, there is no reason to believe any one non-autoclave soil treatment would have more favorable aeration properties than any other non-autoclave soil treatment. Because there was no consistent effect of hollow tine cultivation on the degradation rates of the three pesticides, no general statement about the effect of this treatment on pesticide degradation can be made.

CONCLUSIONS

Comparison of non-autoclaved and autoclaved degradation constants indicates that microbial activity is a significant contributor to the overall degradation of flutolanil and chlorpyrifos in thatch. A similar result was not observed for 2,4-D in thatch. The latter results may be due to the fact that methanol was used as co-solvent to apply all three pesticides. The effect methanol on 2,4-D-degrading organisms has not been documented but other researchers have raised concerns about the potential deleterious effects methanol may have on 2,4-D degraders (Guo et al., 2000). Non-autoclaved flutolanil and chlorpyrifos soil degradation constants were statistically

similar to the non-autoclaved thatch degradation constants for each pesticide. Thus the degradation constant data for these two pesticides is in agreement with the hypothesis that extensive sorption of these pesticides to thatch organic matter effectively shields the pesticides from the presumably large microbial populations present in thatch. The 2,4-D results are not consistent with the results of Roy et al (2001) and Wu et al. (2002a) who reported that minimally sorbed pesticides (i.e., dicamba and metalaxyl, respectively) are more rapidly degraded in thatch than in soil. Repeating this investigation using water as a solvent for 2,4-D in place of methanol may alter the 2,4-D results seen in this investigation. Hollow tine cultivation and vertical mowing did not consistently alter the degradation of any of the three pesticides examined in this study in either thatch or soil. The results of this laboratory incubation study suggest that the aerobic degradation constants (and associated half life values) of tightly adsorbed pesticides obtained in soil incubation studies should be representative of the values that would be obtained in thatch under the same experimental conditions.

Table 4.1. 2,4-D degradation rates constants (k), standard errors (S.E.) and coefficient of variation (CV) values for autoclaved and non-autoclaved thatch and soil that were collected 81 days after imposing one of three cultivation treatments on fairway managed creeping bentgrass.

Media	Cultivation	Autoclaved			Non-autoclaved		
		k	S.E. for k	CV	k	S.E. for k	CV
		— day ⁻¹ —		%	— day ⁻¹ —		%
Thatch	Vertical mow	0.0167	0.00236	14.1	0.0156	0.00345	22.1
	Hollow tine	0.0152	0.00222	14.6	0.0156	0.00247	15.9
	None	0.0114	0.00252	22.1	0.0147	0.00276	18.8
Soil	Vertical mow	0.0118	0.00301	25.5	0.0265	0.00207	7.8
	Hollow tine	0.0083	0.00244	29.4	0.0266	0.00171	6.4
	None	0.0099	0.00248	25.1	0.0278	0.00159	5.7

Table 4.2. Flutolanil degradation rates constants (k), standard errors (S.E.) and coefficient of variation (CV) values for autoclaved and non-autoclaved thatch and soil that were collected 81 days after imposing one of three cultivation treatments on fairway managed creeping bentgrass.

Media	Cultivation	Autoclaved			Non-autoclaved		
		k	S.E. for k	CV	k	S.E. for k	CV
		day ⁻¹			day ⁻¹		
		%			%		
Thatch	Vertical mow	0.0056	0.00083	14.8	0.0081	0.00155	19.1
	Hollow tine	0.0055	0.00076	13.8	0.0059	0.00183	31.0
	None	0.0054	0.00114	21.1	0.0097	0.00142	14.6
Soil	Vertical mow	0.0094	0.00236	25.1	0.0093	0.00134	14.4
	Hollow tine	0.0067	0.00222	33.1	0.0101	0.00085	8.4
	None	0.0079	0.00204	25.8	0.0080	0.00055	6.9

Table 4.3. Chlorpyrifos degradation rates constants (k), standard errors (S.E.) and coefficient of variation (CV) values for autoclaved and non-autoclaved thatch and soil that were collected 81 days after imposing one of three cultivation treatments on fairway managed creeping bentgrass.

Media	Cultivation	Autoclaved			Non-autoclaved		
		k	S.E. for k	CV	k	S.E. for k	CV
		day ⁻¹			day ⁻¹		
		%			%		
Thatch	Vertical mow	0.0078	0.00101	12.9	0.0106	0.00149	14.1
	Hollow tine	0.0081	0.00076	9.4	0.0094	0.00122	13.0
	None	0.0095	0.00088	9.3	0.0109	0.00098	9.0
Soil	Vertical mow	0.0119	0.00233	19.6	0.0123	0.00221	18.0
	Hollow tine	0.0087	0.00274	31.5	0.0138	0.00190	13.8
	None	0.0095	0.00276	29.1	0.0106	0.00168	15.8

Table 4.4. Pesticide degradation rates constants (k), standard errors (S.E.) and the coefficient of variation (CV) for autoclaved and non-autoclave thatch and soil.

Media	Pesticide	Autoclaved			Non-autoclaved		
		k	S.E. for k	CV	k	S.E. for k	CV
		—— day ⁻¹ ——		%	—— day ⁻¹ ——		%
Thatch	2,4-D	0.0144	0.00137	9.5	0.0153	0.00169	11.4
	Flutolanil	0.0055	0.00053	9.6	0.0079	0.00093	11.7
	Chlorpyrifos	0.0084	0.00051	6.7	0.0103	0.00072	7.0
Soil	2,4-D	0.0100	0.00153	15.3	0.0270	0.00104	3.8
	Flutolanil	0.0080	0.00128	16.0	0.0092	0.00056	6.1
	Chlorpyrifos	0.0100	0.00151	15.1	0.0122	0.00112	9.2

Table 4.5. Significance probabilities for the effects of medium, autoclaving treatment, medium X autoclaving, cultivation, and cultivation X medium X autoclave on 2,4-D degradation.

Effect	Comparison of interest	Significance probability
Medium X Autoclave	Autoclaved Thatch vs. Autoclaved Soil	0.0321*
	Non-Autoclaved Thatch vs. Non-Autoclaved Soil	0.0001*
	Autoclaved Thatch vs. Non-Autoclaved Thatch	0.6929
	Autoclaved Soil vs. Non-Autoclaved Soil	0.0001*
Cultivation	Hollow tine vs. None	0.7568
	Vertical mow vs. None	0.3500
Cultivation X Medium X Autoclave	Hollow tine vs. None for Autoclaved Thatch	0.2518
	Vertical mow vs. None for Autoclaved Thatch	0.1249
	Hollow tine vs. None for Autoclaved Soil	0.6468
	Vertical mow vs. None for Autoclaved Soil	0.6251
	Hollow tine vs. None for Non-autoclaved Thatch	0.8086
	Vertical mow vs. None for Non-autoclaved Thatch	0.8371
	Hollow tine vs. None for Non-autoclaved Soil	0.6284
	Vertical mow vs. None for Non-autoclaved Soil	0.6230

* Probability values smaller than 0.05 indicate there is significant difference in degradation rates between the two items being compared.

Table 4.6. Significance probabilities for the effects of medium, autoclaving treatment, medium X autoclaving, cultivation, and cultivation X medium X autoclave on flutolanil degradation.

Effect	Comparison of interest	Significance probability
Medium X Autoclave	Autoclaved Thatch vs. Autoclaved Soil	0.0737
	Non-Autoclaved Thatch vs. Non-Autoclaved Soil	0.2386
	Autoclaved Thatch vs. Non-Autoclaved Thatch	0.0268*
	Autoclaved Soil vs. Non-Autoclaved Soil	0.3977
Cultivation	Hollow tine vs. None	0.4911
	Vertical mow vs. None	0.7769
Cultivation X Medium X Autoclave	Hollow tine vs. None for Autoclaved Thatch	0.9491
	Vertical mow vs. None for Autoclaved Thatch	0.9361
	Hollow tine vs. None for Autoclaved Soil	0.6935
	Vertical mow vs. None for Autoclaved Soil	0.6372
	Hollow tine vs. None for Non-autoclaved Thatch	0.0941
	Vertical mow vs. None for Non-autoclaved Thatch	0.4234
	Hollow tine vs. None for Non-autoclaved Soil	0.0382*
	Vertical mow vs. None for Non-autoclaved Soil	0.3664

* Probability values smaller than 0.05 indicate there is significant difference in degradation rates between the two items being compared.

Table 4.7. Significance probabilities for the effects of medium, autoclaving treatment, medium X autoclaving, cultivation, and cultivation X medium X autoclave on chlorpyrifos degradation.

Effect	Comparison of interest	Significance probability
Medium X Autoclave	Autoclaved Thatch vs. Autoclaved Soil	0.3205
	Non-Autoclaved Thatch vs. Non-Autoclaved Soil	0.1519
	Autoclaved Thatch vs. Non-Autoclaved Thatch	0.0366 *
	Autoclaved Soil vs. Non-Autoclaved Soil	0.2464
Cultivation	Hollow tine vs. None	0.9378
	Vertical mow vs. None	0.6652
Cultivation X Medium X Autoclave	Hollow tine vs. None for Autoclaved Thatch	0.2218
	Vertical mow vs. None for Autoclaved Thatch	0.1972
	Hollow tine vs. None for Autoclaved Soil	0.8477
	Vertical mow vs. None for Autoclaved Soil	0.4983
	Hollow tine vs. None for Non-autoclaved Thatch	0.3371
	Vertical mow vs. None for Non-autoclaved Thatch	0.8864
	Hollow tine vs. None for Non-autoclaved Soil	0.1975
	Vertical mow vs. None for Non-autoclaved Soil	0.5326

* Probability values smaller than 0.05 indicate there is significant difference in degradation rates between the two items being compared.

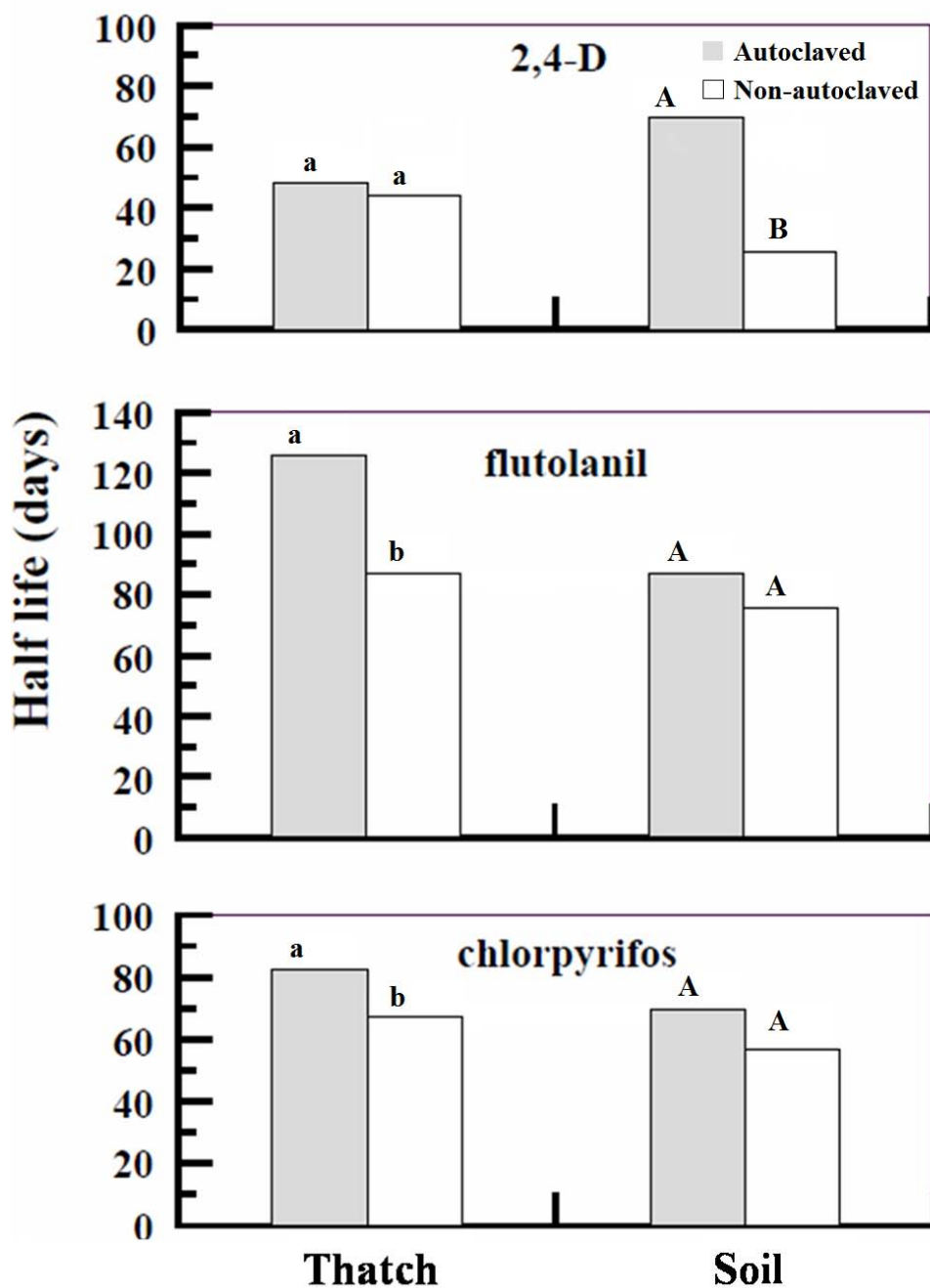


Figure 4.1. Effect of autoclaving on 2,4-D, flutolanil, and chlorpyrifos half life in thatch and soil. Histograms for a specific pesticide that have the same case letter are significantly different from one another at the 0.05 level if letters are different. Histograms having different case letters, and the histograms of different pesticides, should not be compared with one another.

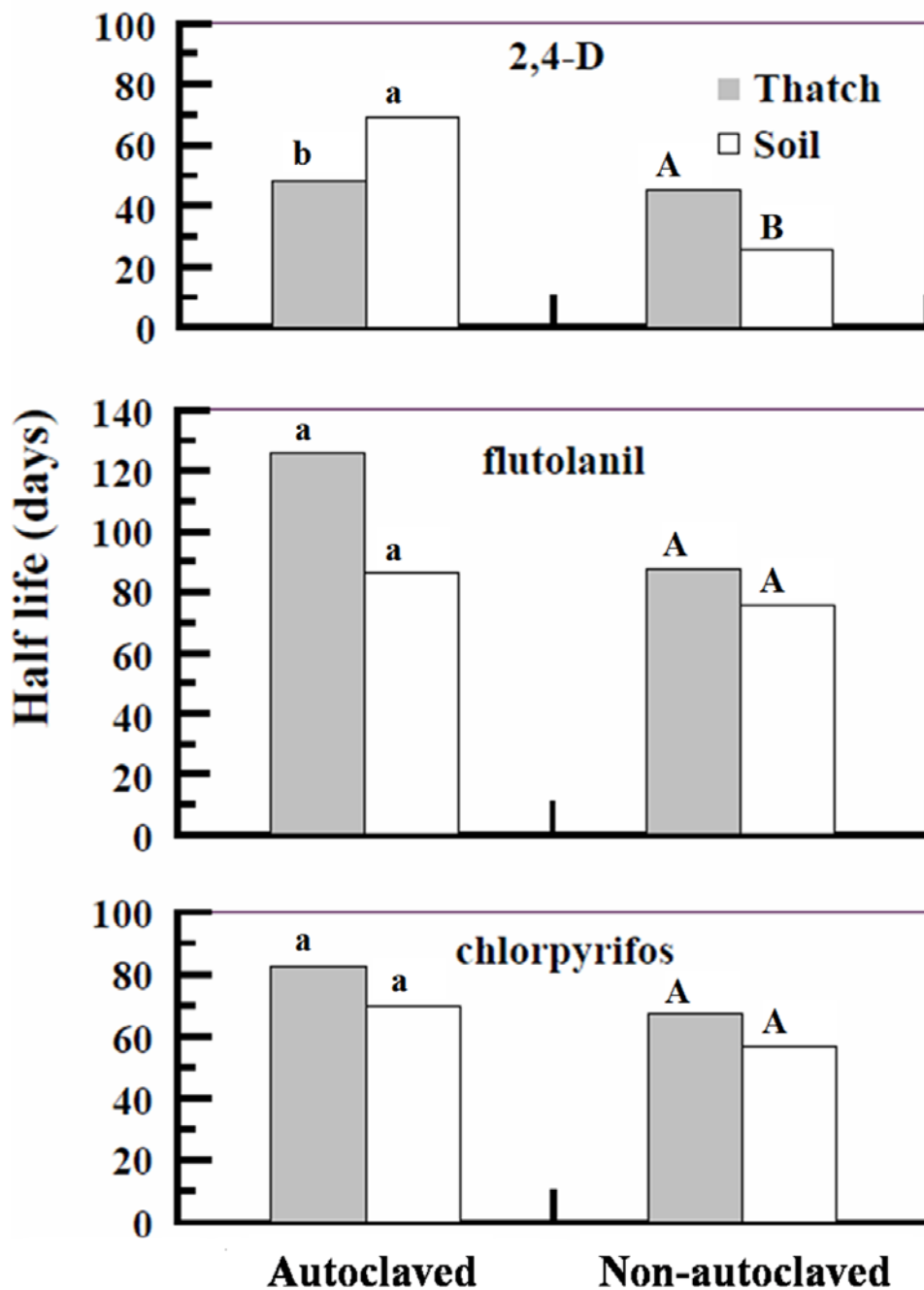


Figure 4.2. Effect of media on 2,4-D, flutolanil, and chlorpyrifos half life in autoclaved and non-autoclaved treatments. Histograms for a specific pesticide having the same case letter are significantly different from one another at the 0.05 level if letters are different. Histograms having different case letters, and the histograms of different pesticides, should not be compared with one another.

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