EFFECTS OF ROUNDUP, GLEAN, AATREX, AND THEIR ACTIVE INGREDIENTS (GLYPHOSATE, CHLORSULFURON, AND ATRAZINE) ON PERIPHYTON COMMUNITIES STUDIED BY USING MATLOCK PERIPHYTOMETER AND BOTTLE TESTS

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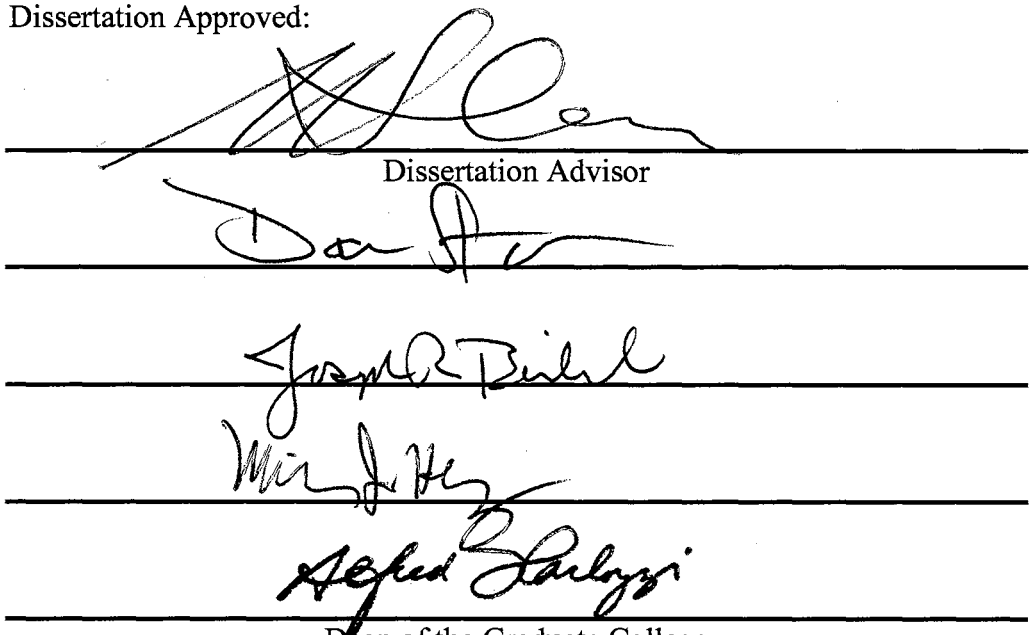

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CHAPTER 1

INTRODUCTION

"Of all our natural resources water has become the most precious. The pollution entering our waterways comes from many sources: radioactive wastes from reactors, laboratories, and hospitals; fallout from nuclear explosions, domestic wastes from cities and towns; chemical wastes from factories. To these is added a new kind of fallout—the chemical sprays applied to croplands and gardens, forests and fields." (Carson, 1962)

The use of pesticides for the improvement of agricultural products started in China around 2500 BC (Timbrell, 1995). The first recorded use of a pesticide was sulfur compounds used to control insects and mites and the first herbicide was iron sulfate used to control the growth of broadleaf plants in 1896 (Timbrell, 1995). In the 1940's, N-naphthalene acetic acid was discovered to possess herbicidal properties, which led to the introduction of 2-4 D, and 4-Chloro-2-methphenoxyacetic acid (MCPA) as weed control in cereal crops (Timbrell, 1995). German scientists synthesized the organophosphate parathion as a nerve agent, which eventually also found useful as an insecticide (Timbrell, 1995). The use of these chemicals for the improvement of food supplies began in earnest after World War II (Timbrell, 1995). Many mass produced pesticides were available at

the time to control both insects and weeds. The insecticide dichlorodiphenyltrichloroethane (DDT) was popular with farmers for the complete eradication of insect pests from the fields. It was also preferred for its persistence in the soil (Landis and Yu, 1995; Timbrell, 1995).

These chemicals had an unexpected side effect that was described for the first time by Rachel Carson in her book "Silent Spring". Carson described the problems with pesticides and the side effects that they can have to harm the ecosystem (Carson, 1962). These side effects were unanticipated and their recognition contributed to the creation of the Untied States Environmental Protection Agency (USEPA) and laws to prevent the loss of non-target organisms (Miller, 1997). The pesticides today have shorter half-life in the environment, higher toxicity to target species, and are less hazardous to non-target organisms, and therefore more environmentally friendly (Miller, 1997). While this is the intended function for these chemicals, it is not always the case that they will behave in the environment as predicted in the lab (Timbrell, 1995).

Runoff of chemicals from agricultural lands is a major source of surface and subsurface pollution today. This is referred to as non-point or diffuse source pollution. The Clean Water Act requires that each state submit to the USEPA an assessment of the waterways found within that state and develop water quality standards to prevent the degradation of those waterways (Gallagher, 1997). These water quality standards are established by the state and are separated into use classification and criteria to protect those uses. Each waterway must be classified according to the intended use, e.g. water supply, agricultural purposes,

recreational use, etc. (Gallagher, 1997). The criteria for each state are typically based upon the federal water quality criteria, of which there are more than 100 pollutants characterized. These 100 pollutants may not be found in all states, and some states have pollutants that are not listed. In this case, the USEPA may decide to assign a criterion value. This is the case with many of the pesticides that are used to improve the food supply in the United States (Gallagher, 1997).

Pesticides must undergo a registration process as directed by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) of 1947. The centerpiece of this legislation is the registration of the pesticide before it can be mass-produced and sold. The manufacturer undertakes the process of registration. Registration can take years before the public can use the product. This process is similar to the registration process enforced by the Food and Drug Administration (Miller, 1997). FIFRA was amended in 1972 to become the Federal Environmental Pesticide Control Act (FEPCA), and control of the registration process was moved from the United States Department of Agriculture (USDA) to the USEPA (Miller, 1997). This allowed the USEPA to control the registration process and keep products from being rushed to market. Although criticized for the length of time the registration process now takes, the USEPA has kept some products from reaching the public because of health concerns (Miller, 1997).

The registration process proceeds from the bench to field-testing. The field-testing process includes fate and transport of the chemical, both active ingredient and surfactants if present (Miller, 1997). Also included are toxicity tests for vertebrate, invertebrate, and in some case plant life that could possibly come into

contact with the chemical. As an outcome of testing, recommendations are developed for the safe application. These recommendations are the result of standardized testing procedures using a group of predetermined test organisms (Miller, 1997). In the case of plant life, the standardized test organisms are unicellular algae, *Scenedemus sp.* and the vascular plant *Lemna sp.* (APHA 1995).

Most of the pesticides that are registered are to improve the quality of the food supply, to increase the yield per acre, to remove disease-causing organisms (Miller and Donahue, 1995). For the agricultural community these chemicals are of great importance. On the other hand, there are unknown elements to these chemicals. The effects upon the total ecosystem where these chemicals are used have not been fully characterized (Miller, 1997).

In 1997 an estimated 1.2 billion pounds of pesticides were used in the United States, which is 22% of the world's pesticide usage (Geisy et al., 2001). Atrazine and glyphosate are the most used herbicides in the United States (Solomon et al., 1996; Geisy et al., 2001). 14,895,000 kg of active ingredient (AI) of atrazine and 6,076,364 kg of AI of glyphosate where used in 2002 (National Agricultural Statistics Service, 2003). Chlorsulfuron has been introduced as a second-generation herbicide and is widely used in the agricultural community to eliminate weeds in wheat.

Herbicides such as chlorsulfuron have been developed for high toxicity, high water solubility, relative short environmental half-life, and EC_{50} values that do not have adverse effects upon terrestrial vertebrates (Gardner et al., 1997; Smith et al., 1996; Sáenz et al., 1997; and Nyström et al., 2000).

The work presented here demonstrates the impact or lack of impact that the herbicides Glean, Roundup, and Aatrex have upon periphyton in streams. These results are important because periphyton are food sources for other organisms in the stream and monitoring the impact upon this group of organisms can give an indication about herbicide impact further along the trophic pyramid.

OBJECTIVES

Objective 1: The primary goal of the study was to determine if environmentally realistic herbicide concentrations in lotic environments (concentrations found in the lotic environment), as defined from literature, inhibit algae growth in lotic ecosystems and/or cause a change to the periphyton community structure. Chlorophyll *a, b*, and $c_1 + c_2$ concentrations can be used to measure this change. Periphyton will be defined as benthic attached algae in this study, similar to the United States Environmental Protection Agency (USEPA) definition (Barbour et al., 1999).

In the process, this study will also assess the applicability of the Matlock Periphytometer as a method to evaluate the effect of herbicides on periphyton community structure in a lotic ecosystem. The Matlock Periphytometer is used to passively introduce a relatively steady supply of herbicide and nutrients into an artificial substrate for growth of periphyton in a natural stream.

Hypothesis: The herbicides, Roundup, Glean, and Atrazine, at environmentally relevant concentrations, will have an adverse effect on algae biomass and or community structure.

Objective 2: To determine whether the commercial formulation of Roundup, Glean, and Aatrex or the active ingredients of these herbicides will reduce periphyton growth rate or reduce the chlorophyll content per mg of fresh weight.

Hypothesis: The commercial formulations, glyphosate, chlorsulfuron, and atrazine and their active ingredients inhibit the growth rate and reduce chlorophyll content of the test organism *Pithophora oedogonia* using a bottle test.

Objective 3: To determine whether mixtures of the active ingredient glyphosate, chlorsulfuron, and atrazine will inhibit the growth rates and/or chlorophyll content of *Pithophora oedogonia* (Chlorophyta).

Hypothesis: The mixtures of herbicide active ingredients will have strictly additive effects on growth rate and/or chlorophyll content in *Pithophora oedogonia* (Chlorophyta).

CHAPTER 2

LITERATURE REVIEW

The use of living organisms, as an indicator of changes in community structure, has been commonplace in environmental impact analyses since the creation of the National Environmental Protection Act in 1970 (Reinke and Swartz, 1999). In aquatic ecosystems the use of periphyton as an indicator of community changes have been well studied (Barbour et al., 1999).

Biological Indicators

Environmental scientists have used organisms as indicators of adverse environmental activity for many years. To determine the extent of a pollution event, sampling plant and animal material can allow proper cleanup to proceed (Barbour et al., 1999). For example, *Escherichia coli* has been used as an indicator of human fecal contamination in freshwater (Droppo et al., 1997), sewage fungus as an indicator of organic pollution (Rutt et al., 1993), and overgrowth of algae as an indicator of phosphate contamination (Haraughty and Burks, 1996). To quantify the impact of pollution in an aquatic habitat, indices of biotic integrity have been developed. These indices of biotic integrity can be regionally specific, can use multiple species, and involve the consolidation of aquatic organism

distribution/diversity into a single value. The aquatic organisms serve as the sentinels of the aquatic community much as the canary did in mines. Using any of these indices will give a general idea of the impact an event has had upon the environment. A well-constructed biotic index can give advance warning that there is a problem (Bourbon et al., 1999).

Periphyton Biological Indicators

Periphyton (attached algae found in streams) (Barbour et al., 1999) are used as bioindicators of pollution for several reasons. The first and primary reason is that periphyton are autotrophs forming the base of food webs (Lowe and Pan, 1996). Because they are a food source in high order streams, all other members of the food web are dependent upon their continued survival. Biotic indices that have been developed based upon diatoms have been shown more precise than those based upon macroinvertebrates (Lowe and Pan, 1996).

The second reason the periphyton community is useful as a bioindicator is because of species richness. In some areas, there can be a large number of species per unit area, each with unique ranges of tolerance to pollutants and nutrients, so the species-based response is unique. In addition, diatoms are more sensitive to organic pollutants than macroinvertebrates (Lowe and Pan, 1996).

Samples of periphyton are easily handled for analysis because typically the sample size is small. Periphyton may be sampled from artificial or natural substrates. These samples can be processed simply in the laboratory by removing the periphyton from the substrates and extracting the chlorophyll. Periphyton is

also relatively easy to identify to genus. The exception to this would be the diatoms, which in most cases require taxonomic expertise (Lowe and Pan, 1996). Periphyton is also a useful indicator of changes in the watershed. Continual monitoring can determine how the community responded to materials introduced into the environment (Barbour et al., 1999)

Invertebrate Biological Indicators

Biological indicators are organisms used to assess the impact of human activity upon the environment (McCahon et al., 1991). Common to the concept of biological indicators is the concept that intolerant organisms will be absent in the presence of the pollutant (McCahon et al., 1991; Cao et al., 1997). The most intolerant (most sensitive) organisms are typically not present in a highly polluted area and, therefore, the area is assigned a lower index value than if tolerant organisms are present. Water quality is rated by averaging the biotic index value for each organism and compared to predetermined ranges for each index (Lenat, 1993, Rutt et al., 1993).

The most widely used indices are those that involve aquatic invertebrates (Dickman et al., 1992;, Kerans and Karr, 1994; Cao et al., 1996; Cao, 1997). Invertebrate genera vary widely in tolerance to pollution. The mayfly orders, for example is one of the most sensitive to changes in dissolved oxygen in water, so is used to identify clean or unpolluted waters impacted by organic material whose decomposition lowers the dissolved oxygen content (Lenat, 1993).

Commonly used invertebrate biotic index (Hilsenhoff Index) has a scale range of 1-10, with 10 being the organism found in the best quality water and 1 the organism found in the worst quality water (Lenat, 1993). This scale reflects the organism placement into one of the three categories (tolerant, semi-tolerant, and intolerant). A minimum of 100 invertebrates is collected. Since co-generic species differ in tolerance, identification to the species level is necessary. The index score is calculated from the relative abundance (Lenat, 1993; Rutt et al., 1993; Medley and Clements, 1998).

Other organisms that can be used for biotic indices include diatoms for heavy metals and organic matter (Nebrat, 1995; Medley and Clements, 1998), fungi for organic matter (Rutt et al., 1993), and fish for toxic and organic matter (Zampella and Bunnell, 1998).

The time of year has to be taken into account and the results will have to be interpreted with that in mind. These indices are seasonally sensitive (Hilsenhoff, 1998) due to seasonal differences in the groups of insect larvae that are present in a stream. For example, during the summer months, some groups of mayflies may undergo a dormancy period because of low dissolved oxygen levels in the water, but are relatively active during the other seasons. Regardless, the Ephemeroptera, Plecoptera, Trichoptera (EPT) index is useful for the detection of impaired waters (Rutt et al., 1993; Hilsenhoff, 1998).

The EPT method is a common use of invertebrates as biological indicators. This method is to collect 100 organisms and separate the Ephemeroptera, Plecoptera, and Trichoptera from the rest of the collection. The number of

different mayfly, stonefly and caddisfly species that are present then determines the index. If the value is above 10 different species then water quality is considered excellent (Lent, 1993; Rutt et al., 1993). Pollutants evaluated in freshwater by this method range from organic wastes from farms, to heavy metals in streams (McCahon et al., 1991; Medley and Clements, 1998).

No one of these indices is universally preferred. Each method has its advantages and disadvantages. The EPT index is used when organisms can be identified to the species level. A more general index such as the Hilsenhoff index may be used in multiple geographic regions around the United States (Hilsenhoff, 1998).

Anthropogenic factors influencing periphyton growth

Effects of toxic substances

There have been numerous publications that have identified the effects of toxic substances upon periphyton communities (Peterson et al., 1994; Gruessner and Watzin, 1996; Graymore et al., 2001; Wendt-Rasch et al., 2003a; Wendt-Rasch et al., 2003b). If herbicides introduced into an aquatic system can cause disruption in species competition (Murphy et al., 2000). The disruption of competition can lead to growth of opportunistic algae such as cyanobacteria (Murphy et al., 2000). Because of the large number, only the papers that summarize the effects of chlorsulfuron, atrazine, and glyphosate will be presented here.

The environmental effects of atrazine have been presented on numerous occasions (de Noyelles et al., 1982; Gruessner and Watzin, 1996; Solomon et al., 1996; Guasch et al., 1997; Graymore et al., 2001). The studies indicate that there are no significant differences in taxonomic composition in streams treated with less than 20 $\mu g\ L^{-1}$ (Gruessner and Watzin, 1996). In streams that had received higher concentrations of atrazine (100-500 $\mu g\ L^{-1}$) a reduction in intolerant species occurred with a shift to smaller cell-sized-tolerant organisms (Graymore et al., 2001). Exposure to atrazine can shift the community composition from filamentous green algae and/or cyanobacteria to dominance by diatoms (Guasch et al., 1997). deNoyelles et al. (1982) also found that after 48 hours of exposure to 20 and 500 $\mu g\ L^{-1}$ of atrazine, periphyton in experimental ponds showed reduced biomass and that the community shifted to tolerant species. Nelson et al. (1999) demonstrated that after 7 days of exposure to 10 $\mu g\ L^{-1}$, chlorophyll *a* content in benthic diatoms was reduced 41-67%. Further, a reduction of 10-50% in total species was found in the microcosms. At 5 $\mu g\ L^{-1}$ there were no significant reductions in either chlorophyll *a* or the number of species present (Nelson et al., 1999).

Austin et al. (1991) stated that glyphosate appears to have little effect on the growth and chlorophyll content of periphyton determining that low concentrations of glyphosate (1-300 $\mu g\ L^{-1}$) had little effect on periphyton biomass and successional patterns of streams. Peterson et al. (1994) determined that only one species of each cyanobacteria and diatoms were sensitive to glyphosate. In a separate study, the colonization of a slide box periphytometer was not dependent

upon the concentration of glyphosate in the stream, but rather the habitat factors (Sullivan et al., 1981).

Evidence for sulfonylurea (chlorsulfuron) herbicides changing the species composition of streams is contradictory. Wendt-Rasch et al. (2003a) determined that environmentally realistic concentrations of metsulfuron methyl (a sulfonylurea herbicide) did not produce significant effects on periphyton communities. Exposure to Metsulfuron methyl at 20μg L^{-1} did not alter the biomass or species composition of phytoplankton in a microcosm (Wendt-Rasch et al., 2003a). (Metsulfuron methyl was the first of the sulfonylurea herbicides, chemically similar to chlorsulfuron). However, when *Elodea sp.* was exposed to 1 μg L^{-1} Metsulfuron methyl, there was reduced root growth and the plants showed signs of nutrient leakage from cell surfaces. Coyner et al. (2001) found that the number of leaves on *Potamogeton pectinatus* declined when exposed to 1 μg L^{-1} and Peterson et al. (1994) found that sulfonylurea herbicides had a stimulatory effect on periphyton at low concentrations.

Herbicides such as chlorsulfuron, atrazine and glyphosate, at environmentally realistic concentrations, may have significant effects on periphyton (Nelson et al., 1999). The response of periphyton to herbicide exposure may depend upon the composition of the community at the time of exposure. Further, stream periphyton assemblages may already reflect the artificial selection from herbicide exposure (Hogland et al. 1996). To determine the effects of atrazine on stream flora, Nyström et al. (2000) used stream water passed through a flume to simulate stream conditions. This also allowed there to be multiple alga species present during

testing. Further, Nyström et al. (2000) showed that *Mougotia sp.* and Cyanobacteria species had lower abundance at the 1.0 μM (200μg/L) level as compared to the other groups present in the study. At higher concentrations of atrazine 1.8μM (360μg/L), community tolerance drops until all members of the community are affected.

Natural Biotic And Abiotic Factors Influencing Periphyton Growth In Streams

Different ecological factors influence the biomass and taxonomic composition of periphyton in lakes and streams. Some of these factors are light (quantity and quality), water temperature, availability of nutrients, turbidity, depth of lakes and streams and flow rate in streams.

Light

The quantity and quality of light is important for photosynthesis. The response to changes in light can account for variation in physiology, population growth and community structure (Hill, 1996). In a stream or lake, irradiance can vary from near zero in turbid lakes to maximum exposure in clear lakes and streams. The effective photoperiod can also vary depending on season, canopy cover, or depth, which reduces photoperiod and irradiance that reaches the algae (Hill, 1996). Powers and Stewart (1987) have indicated that there is a shift in the community structure as irradiance and light duration decreases with the approach of fall and winter. Millie et al. (1992) reported that light conditions were an important consideration in photoacclimated *Anabaena circinalis* and the different pigment

content allowed for differing sensitivity to herbicides. Powers and Stewart (1987) further indicated that Chrysophyta and Cyanophyta become the dominant groups in streams during the late summer, fall and winter.

Chlorophyll *a*, the primary photosynthetic pigment found in all algae, can be significantly higher in streams that have open sites compared to sites that are shaded; if grazing pressure is low. If grazing pressure is high, then biomass as measured by chlorophyll, may not be correlated with light (Hill and Knight, 1988). In lakes, light attenuation is dependent upon absorption and scattering in the water column.

The irradiance of light can influence the structure of the community by favoring high-light adapted species. These species can shift resource allocation away from photosynthetic pigments to enzymes for carbon fixation (Hill and Knight, 1988). The light responses of different taxonomic groups depend upon the amounts of and types of photosynthetic pigments. Richardson et al. (1983) compared irradiances with growth of major phytoplankton groups and reported that chlorophytes do not grow well at low irradiances. Steinman and McIntire (1987) observed that chlorophytes became abundant only at high irradiances in forested streams. Cyanophyta and Bacillariophyceae grow well under low irradiances (Richardson et al., 1983). O'Neal and Lembi (1995) concluded that *Pithophora oedogonia*, has maximum growth under a wide range of irradiances, but *Spirogyra sp.* has maximum growth under only moderate to high irradiances ($500 \mu mol \cdot m^{-2} \cdot s^{-1}$). This would indicate that different taxa of filamentous algae adapt to different light conditions.

Depending on the sampling time there could be a different community present in the stream. If irradiances are low, then diatoms and cyanobacteria would likely be dominant. If irradiances are high, then chlorophytes would likely dominate the stream. Chlorophyceae could also dominate the stream by simply overgrowing the diatoms and cyanobacteria present.

Table 1 shows hypothetical seasonal succession of periphyton in a permanent, shallow, prairie stream in Western Oklahoma. During the winter diatoms are the most abundant. *Gomphomena sp.* is dominant in areas of the stream that have sandy bottoms (Chris Hise, Nature Conservatory, 2000, personal communication) while *Achnanthes sp.*, and *Navicula sp.* have been observed in desert creeks (Fisher et al., 1982). This group is dominant until the middle of spring when *Rhizoclonium sp.* and other chlorophytes become dominant (Power and Stewart, 1987). Green algae will also increase as water temperature increases (Chris Hise, Nature Conservancy, 2000, personal communication).

Table 1. Succession of major periphyton species in streams found in Oklahoma from published literature and personal observations.

Phylum	Genus and species	Time of Year	Reference
Chlorophyta	*Cladophora glomerata*	Late summer, early fall	Hise (Personal Communication 2000)
	Rhizoclonium sp.	Summer, early fall	Power and Stewart (1987)
	Spirogyra sp.	Summer, early fall	Power and Stewart (1987)
	Closterium sp.	Summer, early fall	O'Neal (Personal Communication 2000)
	Mougeotia sp.	Summer, early fall	O'Neal(Personal Communication 2000)
	Pithophora oedogonia	Spring, summer	O'Neal(Personal Communication 2000)
	Stigeoclonium sp.	Summer, early fall	O'Neal(Personal Communication 2000)
	Zygnema sp.	Summer, early fall	O'Neal(Personal Communication 2000)
Chrysophyta	*Gomphomena sp.*	Late fall, winter, spring	Hise(Personal Communication 2000)
	Achnanthes sp.	Late fall, winter, spring	Fisher et al. (1982)
	Navicula sp.	Late fall, winter, spring	Fisher et al. (1982)
Cyanophyta	*Oscillatoria sp.*	Late summer, fall	Power and Stewart (1987)
	Anabaena sp.	Late summer, fall	Fisher et al. (1982)

Filamentous chlorophytes can be found in Western Oklahoma including *P. oedogonia* and, later in the summer *Cladophora glomerata* (Chris Hise Nature Conservatory and Steven O'Neal, Southwestern Oklahoma State University, 2000, personal communications), *Mougeotia sp., Zygnema sp.,* and *Spirogyra sp.*, (Power and Stewart, 1987). Into late summer and early fall, populations of chlorophytes start to decline due to lower light and water temperature. As they begin to decline, the population of cyanophytes begins to increase (Chris Hise 2000, personal communication). The populations of chlorophytes and cyanophytes increase in

concentration in late summer, early fall, primarily due to the decreasing light irradiances and water temperature (DeNicola, 1996).

Water Temperature

Another factor influencing periphyton growth is water temperature. As temperature increases, the rates of respiration and photosynthesis also increase. These rates increase until an optimum point is met where enzyme activities start to decline. Thus, organisms all have optimum thermal limits. As these thermal limits are approached in a stream, most species of algae respond by shifting enzyme and chlorophyll *a* concentrations allowing concentrations of RUBISCO (ribulose 1,5 biphosphate carboxylase) and other Calvin cycle enzymes to increase in response to increasing CO_2 fixation (Thompson et al., 1992). For short periods, *Zygnema sp.* exhibits increased CO_2 fixation at higher temperatures (Mosser and Brock, 1976). It has been suggested that diatoms have a large temperature range (Hustedt 1927-1959, and Patrick, 1977 in DeNicloa, 1996) with the tolerance temperature range between 10-45°C.

Community responses to temperature are similar to those for light with organisms responding to temperatures that are within the zone of tolerance for that species. DeNicola (1996) suggests that temperature response of these organisms could be controlled in the environment of the laboratory, but not the field, making exact determinations of field values harder to obtain (Wetzel, 1983; DeNicloa, 1996).

Nutrients

Primary productivity of algae depends upon light and temperature for the running of the enzymatic reactions but growth depends upon nutrients that are present in the water. In both lentic and lotic freshwater systems, phosphorus and nitrogen may be limiting nutrients for primary productivity. The N/P ratio is important in determining whether a stream is nutrient limited and which nutrient is considered limiting. This can be evaluated using the Redfield ratio. Redfield (1958) hypothesized that growth of phytoplankton would be balanced if the cellular carbon:nitrogen:phosphorus ratio was 106:16:1. This is considered the optimum ratio for determining whether nutrients are limiting. If a body of water has an N:P ratio greater than 20:1 (N to P), then the system is phosphorus limited. If the ratio is less than 10:1, then the system is nitrogen limited (Borchardt, 1996).

In systems that are phosphorus limited, algae gain advantage by storing intracellular phosphorus or can produce alkaline phosphatase that allows phosphate groups to be removed from organic matter (Stewart and Alexander, 1971). In streams that have a high loading of sediment, inorganic and organic solids may carry phosphorus from the surrounding soils.

An abundance of nitrogen and phosphorus can lead to an accelerated growth of algae (Borchardt, 1996). In aquatic ecosystems this can lead to water supplies that are unusable for drinking or recreation. In many instances, cyanophytes could out compete the chlorophytes and the chyrsophytes (Murphy et al., 2000). One reason is the ability of cyanophytes to remove diatomic nitrogen and produce ammonium ion (NH_4^+) (Murphy et al., 2000). The ammonium ion can then be used in the

synthesis of amino acids and cell membranes among other biological molecules (Murphy et al., 2000).

Turbidity

Turbidity in streams and lakes caused by silt may reduce the penetration of light an important consideration in streams with considerable loading of silt from the surrounding watershed. A large precipitation event typically moves silt and other material from erosion increasing the turbidity of the water. Soil particles can contain inorganic phosphorus that may mitigate reduction of light providing a source of phosphorus for primary production causing faster growth (Hill, 1996).

Scouring

Scouring of the streams at times of high water will remove algae that are attached in areas that are exposed. These areas depend upon the stability of the substrate. Patchy removal of periphyton biomass occurs during a high water event, typically in a stream with large boulders or cobbles (Peterson, 1996). Streams whose substrate is mainly sand and gravel will have their periphyton communities devastated by high water flows (Grimm and Fisher, 1989). Those organisms that are located in exposed areas of the stream can be removed by the scouring effect of the sand and small gravel. Those organisms located out of the main flow of the water (areas of bedrock that have depressions) will survive the spate (Peterson, 1996). Runoff also scours the stream removing algae from substratum and

reducing the biomass and primary productivity of the system (Peterson, 1996). If the periphyton community is removed by a spate, typically the first group that will return to colonize the area will be the diatoms (McCormick and Stevenson, 1991).

Grazing of algae by macroinvertebrates and fish

Grazing of algae by macroinvertebrates and fish can lead to a large amount of variation of periphyton density. The quantity and quality of grazers are important in determining the overall primary productivity of a stream. If a stream has a large algal community, it may be due to the removal of grazers by a pollutant (Lowe and Pan, 1996). If there is a significant decrease in primary production it may be due to overpredation by invertebrates and fish (Lamberti, 1996). This overpredation can be an example of cascading trophic interaction by reducing the amount of plant matter the grazing invertebrates and fish population could decline.

Artificial methods for evaluating periphyton communities

Artificial methods for evaluating the periphyton community include non-diffusing and diffusive media periphytometers. A periphytometer is a sampling platform for evaluating the growth of periphyton in a stream. Periphytometers may be floating such as the Catherwood Diatometer (Lowe and Pan, 1996), or submerged as the glass slide box, and anchored into the streambed. Both of these periphytometers use clean glass slides as a substrate for the growth of periphyton. Glass/plastic rod periphytometers are an alternative but using a smooth substrate

such as glass/plastic could lead to the colonization by fewer taxa than a rougher surface (Matlock, 1999a).

Diffusing substrates have an advantage over non-diffusing substrates: they can be used to measure potential or maximum primary productivity (Matlock et al., 1998). Diffusing substrates include agar in clay pots and the Matlock Periphytometer. The results from diffusing periphytometers can indicate the response of periphyton to changes in specific nutrients in a stream or waterway, or can determine if the stream is already at the maximum loading (Matlock et al., 1999a).

The clay pot agar periphytometer has been used for long-term (30-day) studies of the effects of toxic material on stream periphyton (Arnegard et al., 1998). This device delivers material to the stream, and is retrieved and sampled for colonization. Results from these studies indicate a reduction of periphyton colonizing the clay pot as concentrations of metals increase (Arnegard et al., 1998).

The Matlock Periphytometer is designed to promote the growth of periphyton by enriching the local environment with phosphorus and/or nitrogen (Matlock et al., 1999a). By allowing the nutrients to diffuse out of a reservoir to the surface of a growth substrate (glass filter) algae can be recruited to the filter and growth stimulated.

The velocity of the stream can affect the nutrient uptake of algae. In waters that have a velocity in excess of 30 cm/sec, the uptake of phosphorus is reduced by up

to 26%, thus reducing net photosynthesis rate and primary productivity (Borchardt, 1996).

Most standard protocols use only chlorophyll *a* as an indicator of periphyton growth as chlorophyll concentration is highly correlated to algal biomass (Wilhm and Long, 1969; Gustavson et al., 1995; Shehata et al., 1997; Ledger and Hildrew, 1998; Dodds et al., 2002; Guasch et al., 2002). Gustavson et al. (1995) showed that changes in algal biomass exposed to copper correlated with measurement of chlorophyll *a*.

Although all algae contain chlorophyll *a*, chlorophytes also contain chlorophyll *b*, and chyrsophytes chlorophyll *c* in place of chlorophyll *b*. Cyanophyta contain phycocyanin but no chlorophyll *b* or *c*. Thus, analyzing all of the pigments or at least the chlorophyll species reveals more community information. The relative proportion of chlorophyll species can give some indication of dominant periphyton on a growth substrate. For example, if chlorophyll *c* concentration is higher than chlorophyll *b*, this indicates a larger diatom population (Ledger and Hildrew, 1998).

Shehata et al. (1997) determined that chlorophyll species could be used to examine the impact of heavy metals upon Nile River periphyton communities. These periphyton communities shifted from diverse communities to communities' dominated by tolerant algae. The average chlorophyll composition of the community was not statically significantly changed toward any group. Guasch et al. (2002) determined that after 16 days there were reductions in chlorophyll *a* and *b* at 100 $\mu g\ L^{-1}$ of copper with chlorophyll *c* concentrations increased slightly at the

same concentration (Guasch et al., 2002). By using chlorophyll to determine biomass present on a growth surface biomass of consumers can be eliminated (Wilhm and Long, 1969).

Methods of sampling periphyton

Methods of sampling the streams for periphyton will lead to some sample variability. Manual removal involves scraping or washing of periphyton from materials in the stream with the amount of material removed subjective and a decision that varies among researcher. This includes hard substrate (rocks, gravel, wood debris), soft substrates (moss, vascular plants), and loose sediment (Barbour et al., 1999). The results can be imprecise because of the methods used to remove the biomass. In the removal process, if not carefully performed, some periphyton can be lost and unintentionally change test results. Sampling methods will remove an arbitrary fraction of the periphyton within a particular reach.

Behavior of glyphosate, atrazine, and chlorsulfuron in soils and water

The physical/chemical properties of the three herbicides being studied in this experiment are listed in Table 2 and the chemical structure in Figure 1. These values indicate that glyphosate is highly soluble in water and has a high affinity for organic soil particles. Chlorsulfuron and atrazine have lower solubility values in water but do not adsorb well to organic soil particles.

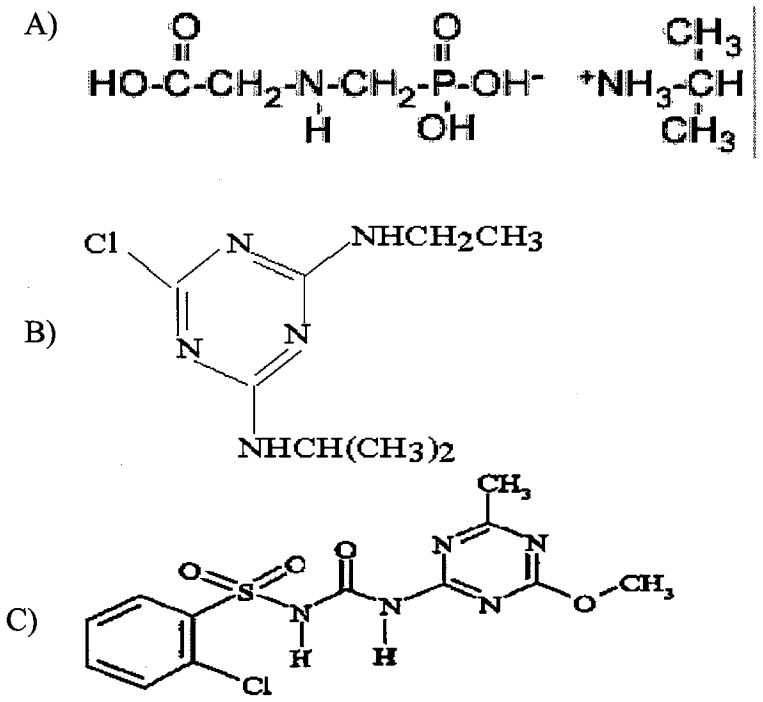

Figure 1. Molecular structure for a) glyphosate, b) atrazine and c) chlorsulfuron.

Table 2. Physical/ Chemical Properties of Glyphosate, Chlorsulfuron, and Atrazine (After Geisy et al., 2000; Solomon et al., 1996; and Strek 1998a)

Chemical	Molecular Weight	Solubility in Water	K_{oc} (affinity for organic carbon)
Glyphosate	169.09 as acid 227.2 as salt	10,000-15700 mg/L	9,000-60,000
Chlorsulfuron	357.8	27.9 mg/L at pH 7 300 mg/L at pH 5	0.69
Atrazine	215.69	28 mg/L	2.34

In soils that are slightly basic, chlorsulfuron has a slight anionic charge. This allows the chemical to move through soil with some ease (Veeh et al. 1994). Atrazine does not adsorb well to soil particles, allowing it to move through the soil matrix to groundwater. Atrazine is one of the most widely used herbicide in the United States, and is commonly found in drinking water supplies, even with its relatively low solubility in water (Allran and Karasov, 2000).

Fate, Transport and Mineralization of Atrazine, Glyphosate, Chlorsulfuron

Atrazine

The fate, transport and mineralization of the active ingredients of herbicides used for this study are important components to consider when examining the impact upon the environment. All of these herbicides have been used in Oklahoma and have the potential to be introduced into waterways (Hogland et al., 1996). Atrazine and its degradation by-products some of the most studied herbicides (Solomon et al., 1996) and have been detected in both surface and ground water samples (Hogland et al., 1996). While the characteristics of glyphosate and chlorsulfuron have not been studied in as great detail as atrazine, there is enough literature to suggest that these herbicides have important impacts upon aquatic ecosystem (Fahl et al., 1995; Geisy et al., 2000).

Organic compounds held by humus undergo mineralization to carbon dioxide. In soils that have a large earthworm (*Lumbercoides terrestris*) population, burrows extending over 6 meters can increase mineralization by binding and holding organic compounds (Farenhorst et al. 2000a). Earthworms' burrows promote the growth of soil bacteria that mineralize organic compounds and increase the cation exchange capacity of the soil.

Farenhorst et al. (2000b) determined that the mineralization rate for atrazine increased over a 68-day period after the initial breakdown of atrazine to hydroxyatrazine (Figure 2, Figure 3). Hydroxyatrazine is formed by the

replacement of the Cl with an OH group at the 2-position on the triazine ring (Lerch et al. 1998). Hydroxyatrazine has a half-life in soils estimated at 165 days, depending upon the soil characteristics and has the potential for accumulating on the soil surface (Lerch et al. 1998). Hydroxyatrazine also has stronger affinity for organic matter than does atrazine (pKa 5.1 to 1.7). Chemical hydrolysis followed by degradation by soil microorganisms accounts for the majority of atrazine breakdown. Addition of organic material increases the rate of hydrolysis. Figures 2 and 3 show the pathways of microbial degradation for atrazine. The soil bacteria break atrazine into either hydroxyatrazine or deisopropylatrazine, depending upon soil conditions (Wackett, 2000). These degradation products are persistent in the soil and are commonly found in water samples (Gong et al., 2001).

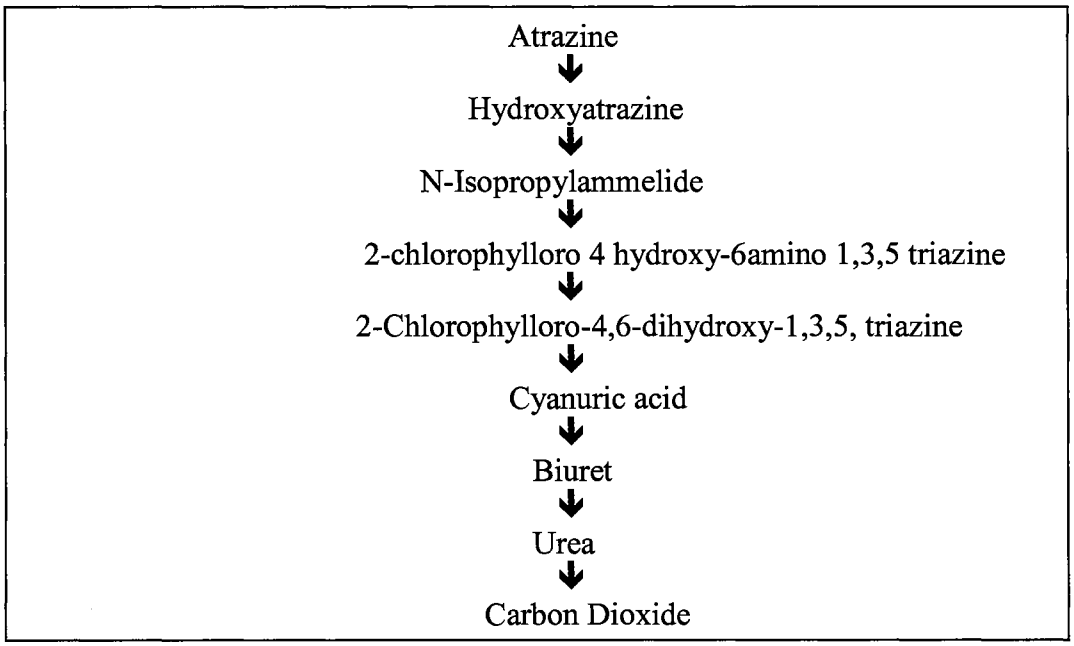

Figure 2. Atrazine mineralization: microbial degradation pathway #1 (Adapted from Wackett, 2000)

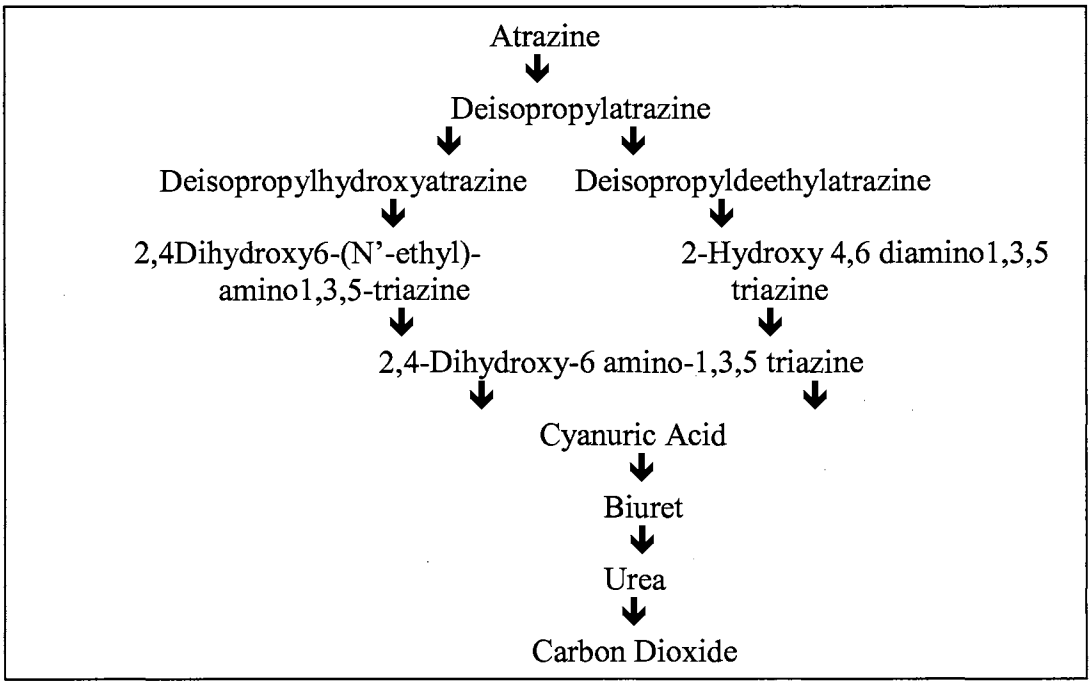

Figure 3. Atrazine mineralization: microbial degradation pathway #2 (Adapted from Wackett, 2000)

The process of photolysis for atrazine is slower in soils with pH values ranging from 5.5-7.5. An excess of either H^+ or OH^- can catalyze the reaction of atrazine forming the various degradation products (Gong et al. 2001). Atrazine and hydroxyatrazine have half-lives of up to 90 days depending upon soil conditions and will take slightly longer, up to 300 days, if the soil is alkaline (pH>8) (USEPA, 2001).

Movement of atrazine through soil horizons to lower strata may be due to preferential pathways in the soil. A preferential pathway in the soil allows for the rapid movement of water from the surface to subsurface. Farenhorst et al. (2000a) determined that atrazine moves through soils to ground water faster with

soils that contain earthworm burrows compared to soil that did not have earthworm burrows. If there is a high density of earthworm burrows, atrazine breaks into the lower horizons of the soil much faster than if there were an intact organic layer to bind the atrazine (Farenhorst et al., 2000A). Atrazine is moderately-to-highly mobile in soils with low clay or organic matter.

Trace amounts of atrazine have been found in drinking water samples and in groundwater samples in a number of states (USEPA, 1994). A five-year survey of drinking water wells detected atrazine in an estimated 1.7% of community water systems and 0.7% of rural domestic wells nationwide. Levels detected in rural domestic wells sometimes exceeded the MCL (Carder and Hoagland, 1998).

Glyphosate

Glyphosate applied to soil begins to break down due to biodegradation controlled by naturally occurring microbial guilds. The major degradation pathway is bacterial removal of the bond between the nitrogen and neighboring CH_2 group, separating this group forming aminomethyl phosphoric acid (AMPA) and glyoxylic acid (Figures 4,5). AMPA is further degraded by the removal of the inorganic phosphate to form a methylamine and then eventually to CO_2 and NH_4^+ (Giesy et al., 2000). Bacteria also mineralize the glyoxylic acid to CO_2. In soils that lack free inorganic phosphate, glyphosate is degraded to sarcosine and then to glycine. These processes can take less than 60 days to complete depending on soil conditions such as pH, moisture, and organic matter content (Giesy et al., 2000).

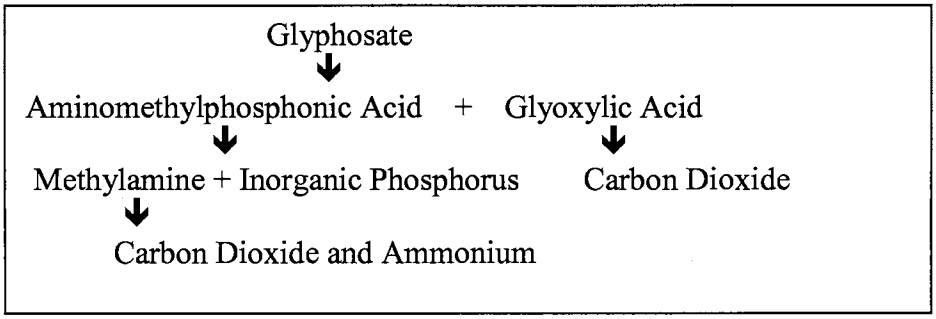

Figure 4. Glyphosate Mineralization: microbial degradation pathway #1 (Adapted from Geisy et al. 2000)

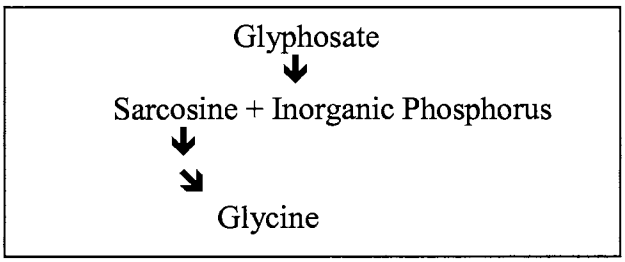

Figure 5. Glyphosate Mineralization: microbial degradation pathway #2 (Adapted from Giesy et al. 2000)

Glyphosate can also become a source of inorganic phosphorus in systems that are phosphorus limited. Bacterial degradation removes the phosphate group resulting in sarcosine. The sacrosine is further degraded to form glycine and ultimately carbon dioxide (Geisy et al., 2000).

When glyphosate is sprayed in water, bacteria break it down into AMPA and CO_2. This process takes from several days to two weeks. Contributing to the removal of glyphosate from water is the adsorption of glyphosate to suspended soil particles in the water (Giesy et al., 2000).

Chlorsulfuron

Chlorsulfuron, a weak acid, is persistent in soils (Strek 1998a; Hultgren et al. 2002). In acidic soils and solutions, chlorsulfuron becomes increasingly protonated (increasing hydrogen ion attachment to negatively charged anions) and uncharged. Chlorsulfuron becomes increasingly soluble in neutral and alkaline soils and can increase the potential for migration into soil pores and ultimately ground water. In soils that have a pH >7.5 the half-life of chlorsulfuron is 10 weeks (Strek 1998a), but chlorsulfuron has been detected in soils in significant quantities more than 12 months after application (Blair and Martin, 1988).

In soils with a pH <7.0, acid hydrolysis, cleaving of the sulfonylurea bridge (the link between the sulfur group and the triazine group)is the first step in the mineralization process (Figure 6). The major products from this first step are chlorobenzenesulfonamide (2-chlorobenzenesulfonamide) and triazine amine (4-methoxy-6-methyl-1,3,5-triazin-2-amine; Strek 1998a). Because there are multiple reaction sites, hydroxylation at any of these appears to be the major degradative pathway (Hultgren et al. 2002). This product, triazine amine is de-methylated and deaminated to form dihydroxy triazine. Dihydroxy triazine breakdowns further by microbial activity to urea and then carbon dioxide (Strek 1998a; Wackett 2001). Increasing soil water concentration increases the potential for biological activity (Hultgren et al. 2002) and available for microorganism decay.

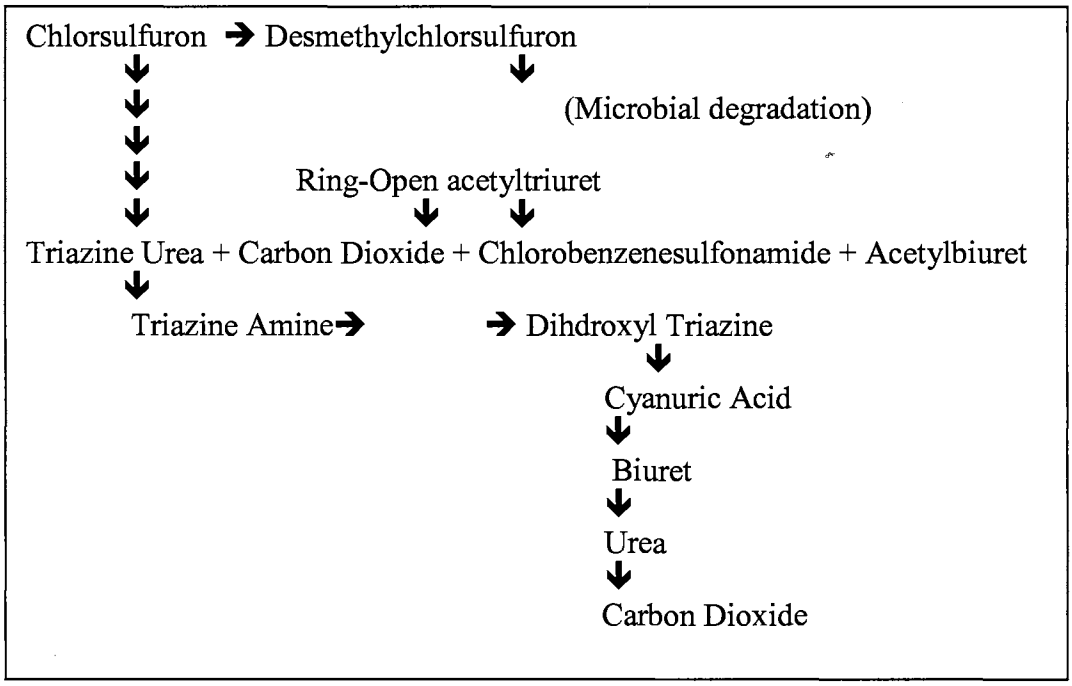

Figure 6. Two pathways for microbial mineralization of chlorsulfuron (Adapted from Strek, 1998a)

Aqueous degradation proceeds in a manner that is consistent with soil processes. The half-life is dependent upon the pH of the water (Strek 1998a; Hultgren et al. 2002). Photolysis of chlorsulfuron occurs at the water surface but is relatively minor in the overall mineralization of chlorsulfuron. When corrected for hydrolysis, the half-life for chlorsulfuron exposed to light in water is >198 days. When compared to aqueous hydrolysis, aqueous photolysis is approximately 8-times slower (26 days-204 days; Strek 1998a). Photolysis has a major role in the degradation of chlorsulfuron on soil and only occurs within the first 0.5 mm of the soil strata. Strek (1998a) showed that there is an 18% difference in the half-life of chlorsulfuron between irradiated and non-irradiated soil (50 days-130 days). When exposed to light chlorsulfuron breaks down into

chlorobenzenesulfonamide, o-desmethyl chlorsulfuron, and triazine amine and triazine urea. Triazine urea is unique to irradiated samples (Strek 1998a).

Microbial breakdown of chlorsulfuron occurs when the chemical has been washed into the soil strata and is dependent upon the microbial mass. The greatest abundance and activity of microorganisms was found in the upper 7cm of the soil (Andersen et al., 2001). The compound is degraded in the soil by hydrolysis, but this breakdown may be prolonged over several weeks to months depending upon the soil conditions. This process decreases when the soil pH is above 6.8 (Obrigawitch et al., 1998).

Introduction of Agricultural Chemicals into the Environment

Introduction of agricultural chemicals (insecticides, herbicides, and fungicides) into the environment is the result of modern cropping practices to produce crops for the growing population; concerns include fertilizers and pesticides (Leboulanger et al., 2001).

Aerial application and tractor-based application to the soil are the preferred methods for herbicide application (Briggs, 1990). Aerial application involves the use of small aircraft to deliver the chemical over a wide area. Other methods include spraying from a vehicle and hand spraying (Briggs, 1990). Use of aerial application for insecticides and herbicides can increase the likely hood of overspray, drift, and dilution (Payne et al., 1992). Aerial application, mainly used for post-emergent applications has the drawback of losing chemical to volatilization off leaf surfaces (Briggs, 1990).

In demonstrating the aerial transport of herbicides, Fletcher et al. (1996) reported that after application of chlorsulfuron to wheat fields, there were losses near by to cherry, canola, and soybean fields when lower than recommended dose of chlorsulfuron were sprayed. Payne et al. (1992) and Riley et al. (1991) reported that concentration of glyphosate is reduced to less than 5% of the original application rate as far as 200 m from the point of origin. Atrazine has had similar reports of drift and dilution of concentration from the source. Introducing herbicides, insecticides, and fungicides into nearby waterways due to wind erosion has also been mentioned as a concern (Payne et al., 1992).

Volatilization of herbicides from previously applied crops is also a concern (Briggs, 1990). The rate of herbicide volatilization is dependent upon air temperature, wind speed, humidity, droplet size and application rate at the time of application. Briggs (1990) gave the following relationship to predict rate of volatilization from vapor pressure of an herbicide

$$\text{Log (E)} = \text{log vapor pressure (Pa)} - 6.62$$
$$= \text{log vapor pressure (mmHg)} - 4.5.$$

These equations can be used to determine the half-life of the chemical on the leaf surface. Briggs (1990) calculated the rate for herbicide volatilization from a flat surface under still air conditions. The units for E are moles cm^{-2} h^{-1} and are a rate measurement. Chlorsulfuron has a volatilization half-life of 12 days, atrazine 10 days, and glyphosate has no value for E because glyphosate has a minimal vapor pressure and E cannot be calculated (Briggs, 1990; Geisy et al., 1998; Solomon et al., 1996). Briggs (1990) reports that even with a low vapor pressure, chlorsulfuron typically does not volatilize from plants or soil surfaces. Once the

herbicide has been volatilized from plant surfaces, it can redeposit as wind velocity decreases (Briggs, 1990). Davidson et al. (2001) suggested that *Rana aurora* (California red-legged frog) populations were adversely affected by aerial transport of agrochemicals.

Under certain conditions, chlorsulfuron, atrazine and glyphosate can adsorb to soil particles and transport with these particles on the wind from one location to another. Another method transport method is on sediment particles in runoff. In either case, the adsorption of chemicals to soil particles is the result of introduction of chemicals by direct soil application. Direct soil applications involve disrupting the soil surface in a manner generally referred to as tilling the soil. Tilling the soil is a method to mix herbicides, fertilizers, and other material to leach into the root zone. These are important for both crop growth and control of unwanted plant growth. Movement of chemicals through the soil by leaching depends upon soil porosity. If the soil is densely packed then herbicides and fertilizers cannot penetrate into the root zone with out tillage (Miller and Donahue, 1995).

Runoff of Chemicals

The type of soil tillage is also important in the runoff from fields. Clausen et al. (1996) reported atrazine concentrations in a Vermont watershed ranged from 1-145 µg/l. The losses were primarily aqueous phase and occurred within three weeks of initial application. In areas where conventional tillage is practiced, atrazine losses to runoff can be due to adsorption to soil particles and has been

measured between 1 to 50,000 µg/l of atrazine (Clausen et al., 1996). In reduced tillage fields, the loss of atrazine is greatly reduced primarily due to the reduction in erosion and runoff (Clausen et al., 1996).

Runoff depends upon several factors. The first is intensity of the rainfall (P) event. The second is the infiltration capacity of the soil (f). Infiltration capacity determines the rate at which rainfall can infiltrate into the soil. If P>f, runoff will occur. Infiltration capacity typically declines through a rainfall event due to increasing saturation (Miller and Donahue, 1995). The soil moisture present in the soil at the time of the rainfall event is a third factor. The higher the soil moisture content the earlier in the rainfall event runoff will occur (Miller and Donahue, 1995). Water that does not infiltrate into soil flows to the lowest point within the watershed. These depressed areas receive water with dissolved or suspended chemicals. Runoff will take nutrients, soil particles, and organic matter and contribute to non-point source pollution of surface waters (Miller and Donahue, 1995).

Ng et al. (1997) reports atrazine was found in surface runoff 79 days after application in Missouri. The concentration of atrazine in runoff is dependent upon the soil moisture, as soil moisture decreases atrazine adsorption increases (Lerch et al., 1998). A rainfall event within a day of application increases loss of atrazine greater than if several days pass between application and rainfall (Ng et al., 1997). Novak (1999) determined that atrazine (as hydroxyatrazine) moved off site in the aqueous state and determined that little was adsorbed to soil particles in runoff. Novak et al. (2001) and Hyer et al. (2001) showed that the loss of

atrazine to a runoff event is low when soils have a particle size of <5mm. The higher cation exchange capacity holds atrazine on site.

Chlorsulfuron is also mobile in soils and is readily transported into groundwater that could be underneath the area being treated. In alkaline soils the mobility of chlorsulfuron is higher than in acidic soils. The solubility increases from 60g m^{-3} at pH 5 to 7000g m^{-3} at pH 7 (Veeh et al. 1994) allowing chlorsulfuron to pass through soil. At acidic pH values, chlorsulfuron undergoes acid hydrolysis and is broken down into chlorobenzenesulfonamide (2-chlorophyllorobenzenesulfonamide) and triazine amine (4-methoxy-6-methyl-1,3,5-triazin-2-amine) (Strek 1998a).

Glyphosate, in contrast, binds tightly to soil particles limiting mobility of the chemical. Cumulative data (Giesy et al., 2000) indicate that less than 1% of glyphosate used was found in runoff. Smith et al. (1996) however found glyphosate approaching 45μg/L in well water of Nova Scotia. The treatment sites were power plant sub-stations and the glyphosate appeared in well water of the surrounding community. This is the only study in which significant levels of glyphosate have been detected in groundwater or runoff (Geisy et al. 2000). Although this is the only study that shows a significant contamination of water due to glyphosate, the European Council has set the glyphosate level in drinking water at 1μg/L, but the USEPA has not yet set a drinking water standard (Geisy et al., 2000).

Mechanisms of Toxicity

Glyphosate

The toxicity endpoints of glyphosate on algae are well documented and presented in Table 3(Geisy et al. 2000).

Table 3. EC_{50} for growth rates from selected algae treated with commercial grade Roundup or glyphosate. Values are taken from 96-hour incubation period data (Adapted from Giesy et al. 2000)

Organism	EC_{50} value mgL^{-1}	Reference
Selenastrum capricornutum	8.0 with Roundup	(LISEC 1989)
Chlorella pyrenoidosa	189.0 with Roundup	Hernando et al. (1989)
Chlorella pyrenoidosa	590.0 with Glyphosate as an acid	Maule and Wright (1984)
Chlamydomonas eugametos	>169.0 with Glyphosate as an acid	Hess (1980)
Scenedesmus subspicatus	166.0 with Glyphosate as a salt	Dengler and Mende (1994)

Glyphosate enters the plant by either direct contact or from soil into the phloem (Leaper and Holloway, 2000). If by direct contact, cationic (phosphate) carriers penetrate through the cuticle of the plant translocating glyphosate into the mesophyll (Satchivi et al. 2000).). Once on the inside of the plant, glyphosate moves to meristematic regions causing cellular death (Sikorski and Gruys 1997).

Glyphosate inhibits the biosynthesis of aromatic amino acids by inhibiting the 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase enzyme (Satchivi et al.2000). EPSP is a key enzyme in the production of aromatic amino acids tyrosine, tryptophan, and phenylalanine. Glyphosate is unusual because it binds competitively with one reactant, (phosphoenol pyruvate) and non-competitively

with a second reactant (shikimate-3-phosphate) (Schonbrunn et. al. 2001). Glyphosate forms a tertiary complex with EPSP and acts as an analog to the PEP oxonium ion. This indicates that the herbicide is a competitive inhibitor of EPSP (Skiorski and Gruys 1997).

The Shikimate pathway proceeds in the following manner: D-Erthose-4-phospate enters from either glycolysis or the oxidative pentose phosphate pathway to interact with phospho-2-keto-3-deoxyheptonate aldolase to form 7-phospho-2-keto-3 deoxy-D-arabinoheptonic acid. In the process there is a loss of 2 protons and 2 phosphate groups to the cytoplasm. 7-phospho-2-keto-3 deoxy-D-arabinoheptonic acid then interacts with 5-dehydroquinate synthesis and NAD^+ to form 5-dehydroquinic acid and H_2O. 5-phosphoshikimic acid is formed from the interaction of 5-dehyroquinic acid with the enzymes 5-deydroquinate dehydratase and skikimate dehydrogenase. To create aromatic amino acids, 5-phosphoshikimic acid is joined to phosphoenol pyruvate, from glycolysis, to form 3-enolpyruvylshikimic acid-5-phosphate. From this intermediate chorismic acid is produced and this structure is the precursor for tryptophan directly and tyrosine and phenylalanine (Goodwin and Mercer, 1990).

Tyrosine, tryptophan, and phenylalanine produced by this cycle are used for the synthesis phenolic compounds (Noble, 1999) and 5-aminolevulinic acid, a chlorophyll precursor. Flavoniod structures can be divided into four different classes, anthocyanins, flavones, flavoids, and isoflavoids and are constructed of two six-carbon ring structures with a three-carbon bridge attachment. One of the

ring structures comes from phenylalanine and the other ring from the malonate pathway.

Anthocyanins and flavoniods are colored pigments found in fruits and flowers and anthocyanins are structural component of carotenoids used as accessory photosynthetic pigments (Goodwin and Mercer 1990). Flavones and flavonols are chemicals that absorb light within the ultraviolet range. They are thought to protect the cell from UV light and have been shown to increase in concentration as UV radiation increases and are thought to have a role in the transport of auxin in the plant (Goodwin and Mercer 1990). Phytoalexins are a group of anti-microbial chemicals used to fight bacterial and fungal invasions. Inhibition of this pathway increases shikimate, thereby increasing the concentration of glyoxylate, which in turn inhibits RuBP carboxylase, stopping carbon fixing (Skiorski and Gruys, 1997; Ma et al., 2001).

The route of exposure in animals is either by dermal contact or ingestion. LC_{50} values for Roundup® range from 4.2 mg L^{-1} to 52 mg L^{-1} for fish and 1.6 mg L^{-1} for amphibians. For terrestrial vertebrates the LD_{50} values range from 4860 to 8064 $mgkg^{-1}$ day^{-1} (Geisy et al., 2000). The primary reason for higher LC_{50} values for terrestrial vertebrates is animals do not have biochemical pathways to produce the aromatic amino acids (Geisy et al., 2000). Because of this the USEPA (1985) classified glyphosate as Roundup® as being virtually non-toxic. Chronic exposures are unlikely due to the rapid degradation of glyphosate in the soil and from the plant. No study to date has indicated a potential human health risk from glyphosate.

Mann and Bidwell (1999) however found the LC_{50} value (96hr) for four species of frogs to range from 8.1 to 32 mg L^{-1} determining that the majority of the toxic effects were from the surfactants used to formulate Roundup®. The EC_{50} values for nonylphenol ethoxylate surfactants alone ranged from 1.1 mg L^{-1} to 12.1 mg L^{-1} and for alcohol alkoxylate surfactants ranges from 5.3 mg L^{-1} to 25.4 mg L^{-1} (Mann and Bidwell, 2001).

Chlorsulfuron

Sulfonylurea herbicides are characterized as broad-spectrum for use at low rates (Sabater et. al 2002). Application rates range from 2-75 g ha^{-1} of formulated material or 15-35 mg ha^{-1} of active ingredient (Sabater et. al., 2002; Fahl et. al., 1995; Coyner et. al., 2001). Fletcher et al., (1996) reports at low application rates, non-target plant demonstrates reduced reproduction rates. When treated at 0.8% of the recommended application rates of chlorsulfuron, soy beans (*Glycine max*) produced 1% of the expected yield, but growth was not affected (Fletcher et al., 1996). When found in aquatic environments, sulfonylurea herbicides have toxic effects on plants. Table 4 summarizes the range of effective concentration values for sulfonylurea herbicides on algae.

Table 4. EC_{50} for growth rates from selected algae treated with sulfonylurea compounds. Values are either growth rates or number of leaves per plant (*Potamogeton pectinatus*) for a 96-hour or 7-day incubation period.

Organism	Herbicide	EC_{50} Value	Reference:
Chlorella saccarophila	Cinosulfuron	104 mg L^{-1}	Sabater et. al (2002)
C. vulgaris	Cinosulfuron	0.21-15.5 mg L^{-1}	Sabater et. al (2002)
Scenedesmus acutans	Cinosulfuron	0.02 mg L^{-1}	Sabater et. al (2002)
S. subspicatus	Cinosulfuron	0.03 mg L^{-1}	Sabater et. al (2002)
Freshwater algae	Chlorsulfuron	0.56 mg L^{-1}	Fahl et. al (1995)
Freshwater algae	Chlorsulfuron	0.02-275.5 mgL^{-1}	Nystrom et. al. (1999)
Potamogeton (7-day) *pectinatus*	Chlorsulfuron	1 μg L^{-1}	Coyner et. al (2001)

Sulfonylurea herbicides (including Chlorsulfuron and Cinosulfuron) are characterized as having low acute/chronic toxicity towards animals and not bioaccumulating in the environment (Sabater et al., 2002). They are weak acids in water and have high soil persistence capabilities (Fahl et al., 1995; Nystrom et al., 1999). Chlorsulfuron inhibits the biosynthesis of branched-chain amino acids valine, leucine, and isoleucine by inhibiting acetolactate synthase (ALS)(also called acetohydroxy acid synthase) causing a halting of cell division and growth (Blair and Martin, 1988; Obrigawitch et al., 1998).

Acetolactate synthase binds thiamine-pyrophosphate and pyruvate to form α-acetolactate or α-aceto-α-hydroxy-butyrate. Both intermediates are necessary to form branched chain amino acids. Loss of branched amino acids causes increased anthocyanin production, stunting the growth in both roots and shoots, and chlorosis and necrosis of leaves (Goodwin and Mercer, 1990). A chlorsulfuron treated plant takes the chemical in through both roots and leaves and translocates it quickly through the vascular system halting cellular growth and causing the

slow death of the plant (Coyner et al. 2001). The effects are similar to the toxic effects of photosystem II inhibitors such as atrazine and the other triazine herbicides (Fahl, et al. 1995).

In freshwater, inhibition of plant growth can occur at levels as low as 200 $\mu g\ L^{-1}$ (Fahl et al. 1995). Coyner et al. (2001) reported reduction of plant length of up to 82% when *Potamogeton pectinatus* was exposed to 1 $\mu g\ L^{-1}$ chlorsulfuron for 4 weeks. Coyner et al. (2001) reported that the number of leaves per plant was reduced by 100% from exposure to 1 $\mu g\ L^{-1}$ chlorsulfuron as compared to the control plants and complete mortality of the plants at 2 $\mu g\ L^{-1}$. Sabater and Carrasco (1997) showed that chlorsulfuron had an EC_{50} value of 54.0-mg L^{-1} when exposed to *Chlorella saccharophiia* for 7 days. Nystrom et al. (1999) reports those EC_{50} values (7-day) for freshwater organism ranges 0.02-275 mg L^{-1} with cyanobacteria and dinoflagellates being the most sensitive to chlorsulfuron. The EC_{50} value for chlorsulfuron was 0.40 mg L^{-1} for a 24-hour bottle test using (*Chorale fascia*) at a pH of 6.5 (Fahl et al., 1995). The EC_{50} value (7-day) at pH 8 was 13.9 mg L^{-1}, about 1000 times higher than the value for pH 5 (Fahl et al. 1995). The increase of EC_{50} value would follow that chlorsulfuron does not dissociate at the higher pH values and therefore is not deactivated (Strek 1998a).

The routes of exposure for the chemical, as listed, are dermal, oral, and inhalation and for vertebrate species the LD_{50} (acute exposure) values range 1363 mg/kg for rodents to >3400 mg/kg for lagamorphs. The route of exposure in humans would be either inhalation or dermal contact. No study to date has

indicated a potential human health risk from chlorsulfuron. (E.I du Pont de Nemours Co., 1990).

Atrazine

Triazine herbicides are primarily soil applied. The roots take up the herbicide, translocating to the growing tips and new leaves of the plant through apoplastic transport. The leaves of the seedlings quickly become starved for food and die. The effect continues as long as there is active atrazine in the soil (Ross and Lembi, 1985).

Triazine herbicides such as atrazine interrupt the flow of electrons through Photosystem II (PSII) of noncylic photophosphorylation (Goodwin and Mercer, 1990; O'Neal and Lembi, 1983). The Photosystem II (PSII) complex is comprised of 25 polypeptides on which the D_1 and D_2 proteins (plastoquinone complex) are at the core with two other major proteins, chlorophyll *a* binding protein and the PsbH protein (Rintamaki et al., 2000; Nystrom et al., 2000). Atrazine binds to the hydrophilic amino acid loop of the D_1 protein. Depending on the composition the loop, binding will be weak or strong. A weak bond causes the Q_B electron acceptor on the D_1 to be replaced by the herbicide (Nystrom et al., 2000). The plastoquinone complex acts as a proton pump that actively moves H^+ from the lumen of the thylakiods to the stroma of the chloroplast (Noble, 1999). The energy for the movement of the H^+ comes from the oxidation/reduction of phaeophytin and plastoquinone (Noble, 1999). From the plastoquinone complex, the electrons are passed to the cytochrome$_{b/f}$ complex (Goodwin and Mercer,

1990) moving $2H^+$ from the stroma of the chloroplast to the lumen of the thylakiod in the process phosphorylating ADP into ATP (Noble, 1999). By replacing this acceptor, the herbicide effectively inhibits the flow of electrons through the Electron Transport System (ETS) (O'Neal and Lembi, 1983; Goodwin and Mercer, 1990; Noble, 1999; Nystrom et al., 2000).

Atrazine may have estrogenic effects upon amphibians. Recently, Hayes et al. (2002) reported at low atrazine concentration (1 $\mu g\ L^{-1}$), larval forms of *Xenopus laevis* (African clawed frogs) developed deformed gonadal tissue. Also, at environmentally relevant concentrations (0.01-200 ppb) male *X. laevis* had 10-fold decreases in testosterone levels (Hayes et al., 2002). At these concentrations, 20% of the larva had multiple gonads or were hermaphrodites. Male larva also had smaller larynges when exposed to low concentrations of atrazine (Hayes et al., 2002). Concentrations of 3 $\mu g\ L^{-1}$ have been shown to cause chromosomal damage in hamster ovarian cell (Newman, 1995) and atrazine has been implicated in breast cancer development by affecting the metabolism of estradiol (Graymore et al., 2001).

Atrazine has been rated slightly to moderately toxic in chronic exposures with vertebrates (Norvartis Co., 2000). Routes of exposure include dermal, oral and inhalation with acute LD_{50} values for atrazine range from 750 $mgkg^{-1}$ to 7000 $mgkg^{-1}$. Chronic values range from LD_{50} 7.5 $mgkg^{-1}d^{-1}$ to 75 $mgkg^{-1}d^{-1}$ for vertebrates. LD_{50} values for other vertebrates are higher. Birds and fish for example, range from 2000 $mgkg^{-1}d^{-1}$ to 5000 $mgkg^{-1}d^{-1}$.

In cell culture studies, there is inconclusive evidence that atrazine is carcinogenic. In mammals, 20% of an oral dose is removed from the body in 72 hours. The other 80% of the atrazine is adsorbed across the gastrointestinal tract and distributed around the body. Of this 80%, the kidneys excrete 65 % and the rest is stored in body tissue (Allran and Karasov, 2000).

Table 5. EC_{50} values for Selected Algae Treated with Atrazine for 96-hour incubation period (Adapted from Solomon et al. 1996)

Organism	EC_{50} Value µg/L	Reference
Chlorella pyrenoidosa	175	Gramlich and Frans,(1964)
C. pyrenoidosa	1000	Ebert and Dumford, (1976)
Ankistrodesmus braunii	60	Burrell et al. (1985)
Scenedesmus quadricauda	500-800	Foy and Hiranpradit, (1977)
S. subspicatus	21	Kirby and Sheahan, (1994)
Chlamydomonas reinhardii	500	Loeppky and Tweedy, (1969)
C. reinhardii	1000	Galloway and Mets, (1984)
Selenastrum capricornutum	200	Parrish, (1978)
Anabaena inaequalis	100	Stratton, (1984)
Microcystis aeruginosa	400	Parrish, (1978)

Table 5 summarizes the EC_{50} values based on 96 hour growth rates for selected aquatic organisms exposed to atrazine. *Scenedesmus subspicatus* has an EC_{50} of 21 µg L^{-1} (Kirby and Sheahan, 1994). At the other end of the spectrum *Chlorella pyrenoidosa* has EC_{50} range from 175 µg L^{-1} to 1000 µg L^{-1} (Ebert and Dumford, 1976).

Surfactants

Surfactants are used to carry herbicides across the cell membrane of plants (Paveglio et al., 1996), and include alcohol ethoxylate, linear alkylbenzene, and

alkylphenol ethoxylate (Dorn et al., 1997; Jorgenson and Christoffersen, 2000; and McLeese et al., 1981). Surfactants have been implicated in some toxicological studies, as a major contributor to the overall toxicity of commercial herbicides (Mann and Bidwell, 1999a, b) although Dorn et al. (1997) states that alcohol ethoxylate has no observable effects on *Myriophyllum aquaticum* at 5.15 mg L^{-1}. Using linear alkylbenzene Jorgenson and Christoffersen (2000) determined that 7-day growth inhibition (EC_{50}) for *Microcystis aeruginosa* as 0.5-0.8 mg L^{-1} and for *Chorella pyrenoidosa* the 7-day EC_{50} values ranged from 10-29 mg L^{-1}. Hense et al. (2003) determined an EC_{50} (72 hr) of 0.87 mg L^{-1} using nonylphenol with *Scenedesmus subsicatus* in bottle tests. Hense et al. (2003) also determined using outdoors microcosm cyanophytes had higher population counts when exposed to high concentrations of nonylphenol. Jorgensen and Christoffersen (2000) concluded that cyanobacteria are more sensitive to household surfactants than are the chlorophytes. Belanger et al. (2002) also concluded that algal communities are not altered by exposure to alkylbenzene sulfates with the No Observable Effects Level (NOEL) ranging from 1.1-27 mg L^{-1} at 7-days. Simenstad et al. (1996) found that when an anionic surfactant (X-77) was applied to a mudflat, aquatic invertebrates and marine phytoplankton were more sensitive to the alkylphenol ethoxylate surfactants than to the herbicide glyphosate. Lewis (1990) determined that alkylphenol ethoxylate has a Lowest Observable Effect Concentration (LOEC) on chlorophyte algae of 20-50 μg L^{-1}.

Several researchers (Dorn et al., 1997; Belanger et al., 2002; Paveglio et al., 1996; and McLesse et al., 1981), have shown that for all groups of surfactants

there is increasing toxicity with increasing number of carbons. These carbons are located on a chain that can have between 10 and 16 carbon groups. These carbon groups will then have anywhere from 1-17 ethoxylate groups (Paveglio et al., 1996).

Surfactants are susceptible to degradation by both microbial communities and by photolysis (McLesse et al., 1981). If the degradation of surfactants is microbial in nature, the ethoxylate chain is removed as either a series of ether cleavages or by terminal alcohol oxidation followed by the removal of the carboxyl group that is formed (McLeese et al., 1981). In either case, the ethoxylate groups are removed and only the parent compound remains (see Figure 7.). With the alkylphenol ethoxylate surfactants the parent compound is an alkylphenol ring (Ball et al., 1989)

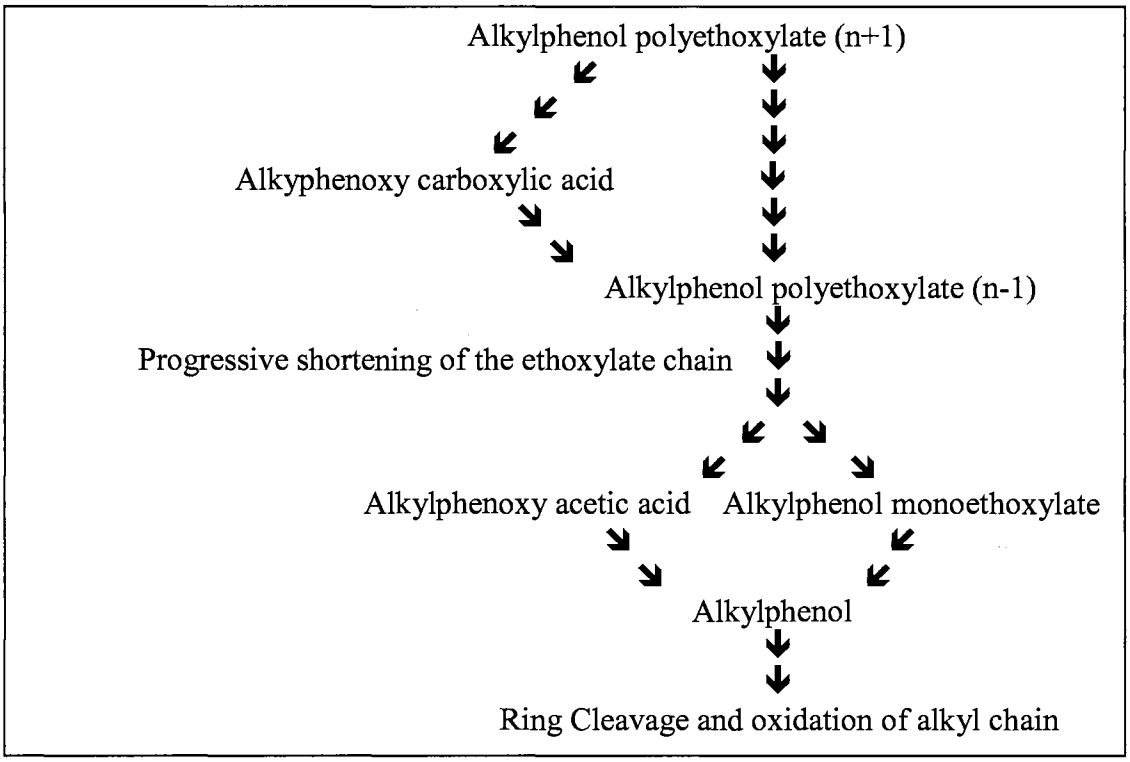

Figure 7. Degradation pathway for alkyphenol polyethoxylates (adapted from Ball et. al, 1989)

CHAPTER 3

MATERIALS AND METHODS

Introduction

One of the objectives of this study was to determine the impact of environmentally relevant concentrations of the herbicides Glean, Aatrex and Roundup upon periphyton communities. This objective was performed at the USDA-ARS Hydraulics Laboratory Research Station on Lake Carl Blackwell, (Stillwater, Oklahoma) utilizing an outdoor flume under controlled flow conditions. The Matlock Periphytometers were initially filled with a nutrient solution consisting of 20 mg L^{-1} $NaNO_3$ and 20 mg L^{-1} $Na_2HPO_4 \cdot 7H_2O$. The periphytometers were allowed to recruit a standing crop of periphyton for a one-week period. The herbicides Glean, Aatrex and Roundup were then injected into the Matlock Periphytometer to passively diffuse through glass filter substrate of the Matlock Periphytometer into the flume to determine any disruption of community structure by measuring chlorophyll species.

Matlock Periphytometer

The Matlock Periphytometer was used to evaluate herbicide toxicity to periphyton communities (Figure 8). The Matlock Periphytometer consists of a 250-ml reservoir, a 0.45-μm nylon membrane, a Whatman 940 glass fiber filter, and a cap with a 22-mm hole. Modifications to the Matlock et al. (1998) design included substitution of an amber Naglene bottle to prevent photolysis of herbicides (Strek, 1998a; Lerch et al., 1998; Geisy et al., 2000) and addition of a rubber septum through which herbicides could be injected to the reservoir after initial incubation. To introduce the septum, 14-mm diameter hole was cut into the surface of the reservoir using a cork borer. The septum was inserted into the hole and sealed into place with silicon sealant (Liquid Nails® clear silicon sealant). The sealant was necessary to prevent leakage from the reservoir while being filled in the field.

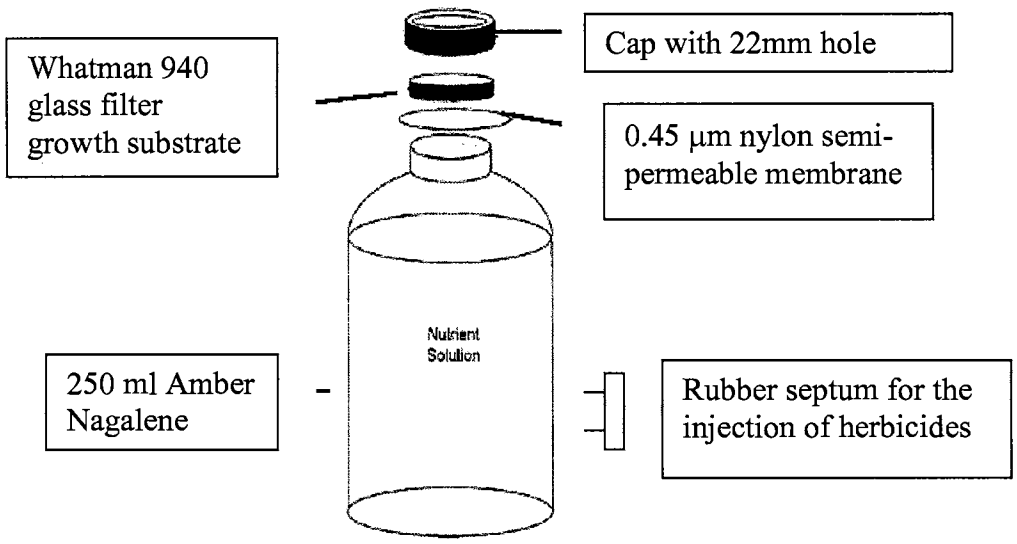

Figure 8. Structural diagram of Matlock Periphytometer bottle changes include the addition of a rubber septum for herbicide injection and an amber bottle to reduce herbicide loss (adapted from Matlock et al. 1998)

Results of preliminary experiment with the Matlock Periphytometer

Preliminary experiments determining the feasibility of using the Matlock Periphytometer (MP) for herbicides were addressed. The question of whether the herbicides would diffuse through the 0.45-μm membrane was investigated. The MP reservoir was filled with a 1 % Roundup® solution and placed into a 2 liter beaker filled with de-ionized water. The diffusion of Roundup® was measured indirectly as conductivity in the MP reservoir using Oakton Model WD 35607-10 conductivity meter. Measurements inside of the periphytometer were done by carefully removing the cap and glass filter. Conductivity was measured initially every two hours for the first eight hours, then every 24 hours after, until equilibrium was achieved at 120 hours as describe by Matlock et al. (1998). This procedure was repeated a second time using water from Deer Creek, Custer County, Oklahoma.

Rate of diffusion through the membrane can be calculated using Fick's Law:

$$J=-D*\Delta C/\Delta x$$

where J is the mass of the material diffusing through a membrane. D is the diffusivity constant over a given time. ΔC is the assumed differences concentrations of glyphosate solution inside and outside the membrane and Δx is the distance across the membrane (Matlock et al., 1999a).

Results in Figure 9 shows the herbicide Roundup diffuses through the membrane at an exponential rate. From time 0 hrs to 120 hrs the amount of herbicide that diffuses across the membrane is consistent. The coefficient of diffusion was calculated at $0.21 cm^2 h^{-1}$ (r^2 =0.95) by non-linear regression

($y= 2594.8^{-0.002x}$). Matlock et al. (1998) calculated the coefficient of diffusion as $0.40cm^2\ h^{-1}$ for potassium chloride using a similar method.

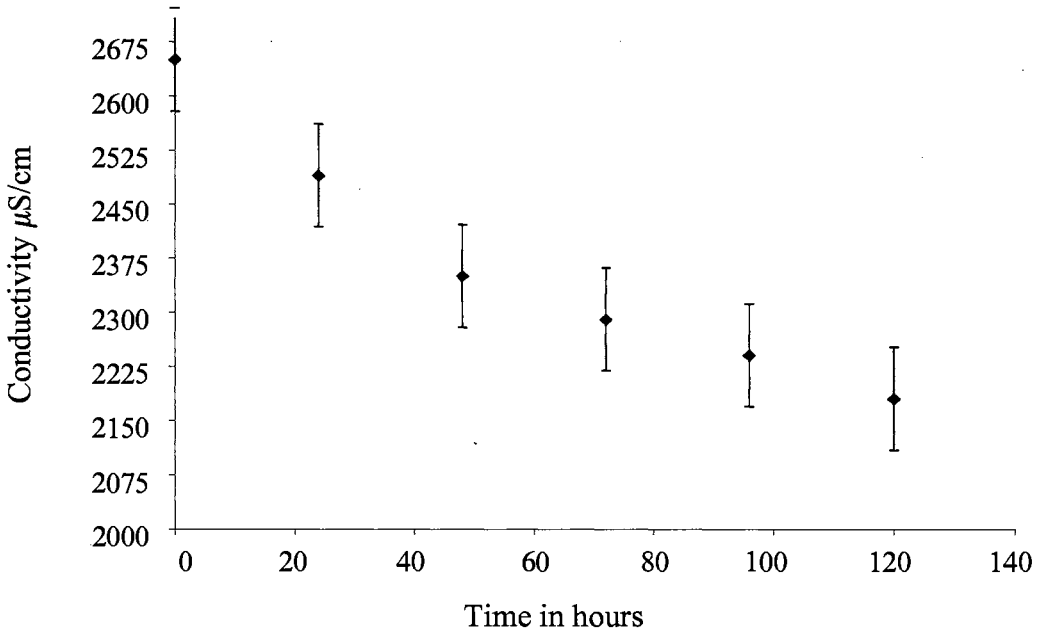

Figure 9. Change in conductivity within the Matlock Periphytometer due to diffusion. The error bars represent standard errors of the mean.

Herbicide Formulations, Active Ingredient

Active ingredient glyphosate in the form of Rodeo® was purchased locally. Technical grade (active ingredient) atrazine and chlorsulfuron were provided by the manufacturers (Novartis Chemical Company Greensboro, North Carolina, and E.I.DuPont de Nemours Chemical Company Wilmington Delaware, respectively).

Stock solutions of atrazine and chlorsulfuron were prepared by dissolving the wettable powder in de-ionized water to produce 6-mg L^{-1} solution of atrazine and 500-mg L^{-1} solution of chlorsulfuron. Test concentrations of glyphosate were prepared from Rodeo® liquid concentrate, diluted with de-ionized water to the stock concentration of 1000-mg L^{-1}. Initial nominal concentrations of active ingredients of the herbicides were 100, 500, 1000 mg L^{-1} of glyphosate, 25, 50, 75, 100 μg L^{-1} of atrazine, and 0.5, 5.0, 50.0 mg L^{-1} of chlorsulfuron were prepared by dissolving a wettable powder or dilution of concentrate into de-ionized water. These concentrations have been reported in the literature have been found in water samples throughout the Mid-West (Hayes et al., 2002). Initial and final concentrations of the herbicides and the effects of the de-ionized water in the bottles were not measured.

Herbicide Formulations, Commercial Formulations

The commercial formulations used were identical to formulations used for field-testing. Experimental concentration of herbicides are similar to concentrations reported previously from both field and laboratory studies (Coyner et al. 2001; Giesy et al., 2000; O'Neal and Lembi, 1983). A locally purchased surfactant X-77 was prepared by diluting the concentrate solution to 1:1000, simulating the lowest amount of surfactant added to the herbicide Rodeo®.

Stock solutions for the commercial formulation portion of the experiment were prepared using 2 times the tank mixture concentrations specified by the manufacturer. Aliquots of 1 to 5 ml were then taken from the stock solutions and

were injected into the reservoirs of the Matlock Periphytometer. Table 6 shows the concentrations of the herbicide treatments from the stock solution calculated using the standard volume/concentration equation from assumed stock solution concentrations as indicated on the herbicide labels.

Table 6. Nominal Concentration of active ingredient of Aatrex, Roundup and Glean (commercial available formulations) used in field and bottle experiment as calculated from stock concentrations. Stock concentrations are 2X the manufactures recommended application rates for each commercial herbicide.

Amount of solution (ml/250ml)	Atrazine in Aatrex mg L^{-1}	Chlorsulfuron in Glean mg L^{-1}	Glyphosate in Roundup mg L^{-1}
0	0.000	0.0	0.0
1	0.003	7.8	7.8
2	0.006	15.6	15.6
3	0.009	23.4	23.4
4	0.012	31.2	31.2
5	0.015	39.0	39.0

Periphytometers without addition of herbicide were used as a positive control, and periphytometers filled with a 1mg L^{-1} $CuSO_4$ solution were used as a negative control (Arnegard et al., 1998). The resultant growth on the positive control periphytometers is used for comparison to treatment periphytometers. Negative controls were used to determine if growth could be inhibited.

Field Study

The field evaluation of the Matlock Periphytometer was performed at the USDA Agricultural Research Service Hydraulics Laboratory in Stillwater, OK during the months of June through October 2002. Each herbicide treatment was tested at least twice during this period with a minimum of six (6) replicates for

216 replicate analyses as a split-plot ANOVA method using SAS (2000). The numbers of replicate periphytometers (Appendix C) were determined using Stein's Two-Stage sample determination method (Steele et al., 1996). The periphytometers (Figure 10) were attached to a metal rack approximately 2.5-m (X) 1-m with parallel strands of metal used to support the testing bottles. Floats constructed of 2.5-cm PVC piping to which the metal rack is attached. Each date of analysis was treated as a separate plot leading to different control values for each date.

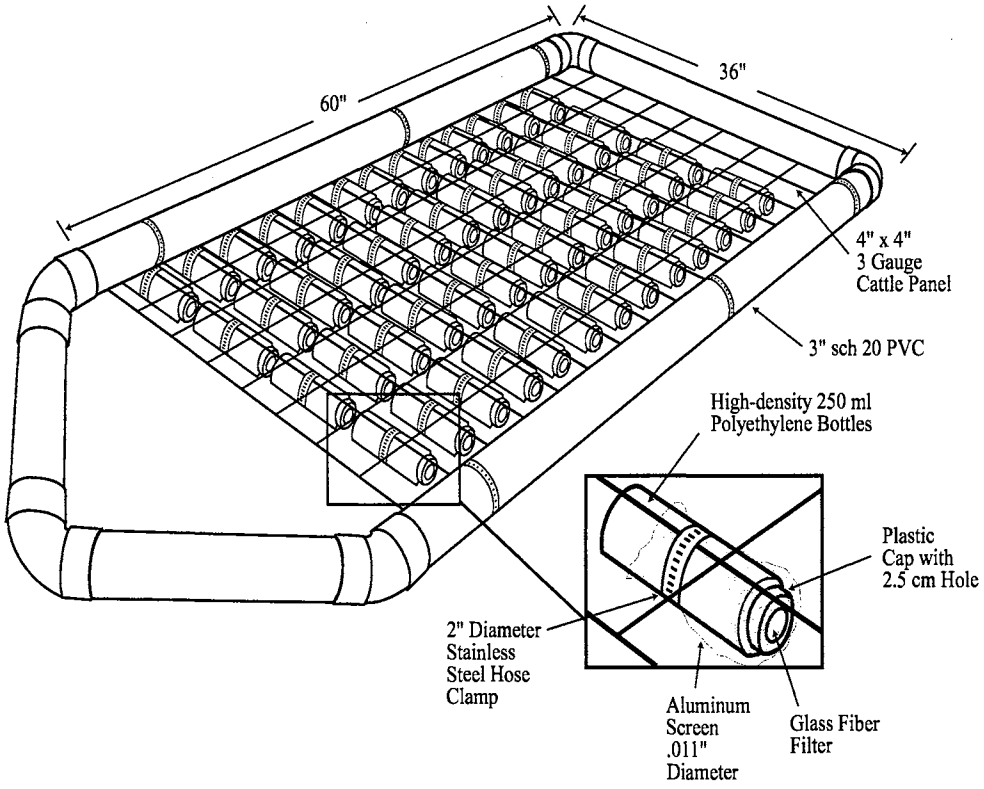

Figure 10. Schematic of the Matlock Periphytometer within the steel frame, which is then placed into a stream. (Matlock et al., 1998)

Water was diverted from Lake Carl Blackwell into a pre-existing concrete flume, (United States Department of Agriculture-Agriculture Rerseach Station: Hydraulic Engineering Research Unit Laboratory, Stillwater Oklahoma), 15-m in length and having a trapezoidal shape with a 0.64-m floor, top width of 1.5-m, and a depth of 1.2-m. A weir was constructed of 1.9-cm inch plywood with a 1.2-cm inch pipe fitted with a valve to control flow (the valve was placed three inches above the bottom of the plywood) and the weir was placed at the 15-m mark downstream on the flume.

The nutrient solutions placed into the periphytometer (Figure 8) were equivalent to 20 mgL^{-1} for both nitrate-nitrogen and phosphate-phosphorus. Mixing appropriate amounts of $NaNO_3$ and $Na_2HPO_4 \cdot 7H_2O$ into de-ionized water, stock solutions were prepared. Concentrations were measured using a HACH 2000 spectrophotometer and methods 8171 and 8048 (Haraughty and Burks, 1996). This solution was then added to the periphytometer in the field, filling each bottle to its maximum. The lake water was evaluated for Nitrate-Nitrogen and Phosphate-Phosphorous (as reactive PO_4) concentration by using the same methods as above.

Periphytometers racks (Figure 10) were kept in the stream for a two-week period. The first week periphytometers were allowed to recruit a standing crop of periphyton. At the beginning of the second week, the periphytometers were treated with varying amounts (1-5 ml) of the herbicide stock solution injected through the rubber septum using an 18-gauge hypodermic needle with a 50-ml syringe with herbicide concentrations randomly placed through out the frame.

The time scale selected was chosen to simulate a periphyton community challenged by a transient herbicide contamination event.

After a 14-day stream exposure, the glass filter was removed from each periphytometer, wrapped in aluminum foil, and placed on ice for transport to the laboratory. At the laboratory, chlorophyll samples were extracted and analyzed using APHA (1995) guidelines. Filters were ground and extracted using cold 90% acetone over $MgCO_3$ for a period not exceeding 24 hours. The extracted samples were centrifuged at 1000 rpm for 10 minutes in 15-ml glass centrifuge tubes to clarify the supernatant. The supernatants were analyzed using a Milton Roy VIS spectrophotometer with a maximum 8-nm bandpass, at 664 nm for chlorophyll *a*, 647 nm for chlorophyll *b*, and 630 nm for chlorophyll *c*. Because the bandpass is > 5 nm, the values for chlorophyll's *b* and *c* may have larger errors than desired. This error is due to small changes in wavelength between pigments and ratios between the chlorophyll *b/a* and *c/a*.

The samples were also read at 750 nm to obtain an interference value to determine the scattering due to suspended material (Jeffrey and Humphrey, 1975). Chlorophyll *a*, *b*, *c* and total chlorophyll where determined using the following equations (Jeffrey and Humphrey, 1975):

Chlorophyll *a* (mg/L extract)=11.85*A664-1.54*A647-0.08*A630

Chlorophyll *b* (mg/L extract)=21.03*A647-5.43*A664-2.66*A630

Chlorophyll *c1* + *c2* (mg/L extract)=24.52*A630-7.7*A647-1.64*A664

where the absorbance of each chlorophyll species is multiplied by the extinction coefficient for that chlorophyll species relative to each other.

The interference value was subtracted from each filter value and total chlorophyll was calculated by adding together the corrected values of extract. To determine the chlorophyll per unit area, the chlorophyll values were divided by 6.6 cm^2 the cross sectional area of the filter. Chlorophyll per unit area was used as a measure of biomass recruited to the filter.

Bottle Tests for Toxicity of Roundup, Glean, and Aatrex

Further, bottle tests for determining toxicity of the herbicides Glean, Aatrex and Roundup (both commercial and active ingredients) were designed by using the APHA (1995) guidelines. Using the bottle tests the effects on growth rates of *Pithophora oedogonia* were observed. Growth rates were determined by the change in fresh weight for the seven-day test period.

Bottle tests studying effects of commercial herbicides Roundup, Aatrex and Glean and active their ingredients (glyphosate, atrazine, and chlorsulfuron) were performed. *P. oedogonia* was selected because it is easy to culture and provides a potential model organism (filamentous algae) that can be used for toxicity testing. *P. oedogonia* also is found throughout the United States in low flow streams and lakes (O'Neal and Lembi, 1983). EPA and APHA standards for bottle toxicity testing uses either a unicellular alga (*Scenedesmus sp.*) or a vascular plant *Lemna sp.* (APHA, 1995). Filamentous algae have not been widely used as indicators of toxicity, but O'Neal and Lembi (1983) used various filamentous

algae to determine long-term (45 days) effects of simazine (a triazine herbicide) on growth rate and chlorophyll *a* content.

Steven O'Neal (Southwestern Oklahoma State University) provided the *P. oedogonia* (SWOSU culture IN-04), which were maintained under axenic conditions using modified Cladophora II medium (Table 7).

Table 7. Modified *Cladophora* II Medium (O'Neal and Lembi, 1983). All solutes were dissolved in deionized water to the appropriate final concentrations.

Compound	Stock concentration	ml of stock/L
NaEDTA	0.05 g /500 ml	10 ml
Fe-citrate/citric acid	0.3 g /500 ml	10 ml
$CaCl_2$ $2H_2O$	4.29 g /500 ml	10 ml
$MgSO_4$	1.83 g / 500 ml	10 ml
$NaHPO_4$	0.64 g/500 ml	10 ml
$NaNO_3$	5.09 g / 500 ml	10 ml
$NaHCO_3$	12.50 g / 500 ml	8 ml
KCl	1.72 g / 500 ml	10 ml
Vitamin B12	0.5 g / 500 ml	5 ml
Trace Metals*		1 ml of total solution
$MnSO_4$ H_2O	0.42 g / 500 ml	
$NaMoO_4$ $2H_2O$	0.013 g / 500 ml	
$ZnSO_4$ $7H_2O$	0.29 g / 500 ml	
H_3BO_3	0.77 g / 500 ml	
$CuSO_4$ $5H_2O$	0.063 g / 500 ml	

*To prepare *Cladophora* solution combine 100 ml of each trace metal stock solution and use 1 ml of that solution for each liter of solution.

Algae were separated into 10-30 mg of fresh weight mats weighed using a Denver Instrument analytical balance Model M-220D and randomly placed into 250ml culture flasks with 100ml of growth medium. Eight replicates of the test cultures were prepared and the stock herbicide solution was placed into the flasks by serial dilution. These cultures were then placed into a growth chamber for the

seven-day incubation period. Growth rates were determined by measuring mg of fresh weight at the end of a seven-day incubation period. The seven-day period was based on recommendations of Mike McKee, Monsanto Chemical (personal communication), Sally Leva, DuPont Chemical (personal communication), and the APHA (1995) seven-day bottle test protocol. Growth conditions were kept constant by culturing the algae in growth chambers at 20° C with a 12/12-hr light/dark cycle. The photon density flux averaged 76 μmol/m^2s, measured with a Li-Cor meter model LI-189 with a cosine-corrected sensor Model LI-191SA.

Chlorophyll *a* content of the mats of *P. oedogonia* was determined using the APHA (1995) method and measured as previously described. Pheophytin was measured by adding 1 drop of 1N HCL and measured at 665nm.

Chlorophyll *a* content of the mat was determined by the equation:

μg Chl *a*/mg fresh weight of sample= (Chl *a*-pheophytin)*145.585/final weight.

The specific growth rate is calculated as:

$$(\mu\ d^{-1}) = \ln (W_f - W_i)/\ 7\text{days},$$

where $\mu\ d^{-1}$ is the growth rate per day, and W_f and W_i are the final and initial fresh weights respectively. It was assumed the growth of *P. oedogonia* was exponential throughout the testing period (APHA, 1995).

Mixture concentrations of active ingredients were based on by the seven-day EC_{50} values for each of the individual herbicides. The seven-day EC_{50} value was calculated by determining the 50^{th} percentile for growth rate of the control values using Linear Interpolation Method software (USEPA, 1988). The EC_{50}

concentrations for the active ingredients are 0.06 mg L^{-1}, for atrazine, 27.57 μgL^{-1} for chlorsulfuron, and 67.10 mg L^{-1} for glyphosate. The EC_{50} value of atrazine was extrapolated using non-linear regression (Graph Pad software). The ranges of concentrations were not sufficiently spread to determine the EC_{50} value using the Linear Interpolation software. The amount of active ingredient added to each flask was equivalent to its EC_{50} value and added to each flask along with the algal mat by micropipets.

The mixture of herbicides was used to simulate the practices that occur in the agricultural industry to kill difficult weed species in the field (Roger Penner, personal communication). Marking (1977) states that toxic chemicals can have an additive, antagonistic, or synergistic effect on organism being tested. To determine which of these characteristics were being observed, the following equation was employed:

$$A_m/A_I + B_m/B_I = S,$$

where A and B are the chemicals being tested, I is the individual EC_{50} for A or B, M is the EC_{50} value for the mixture of A and B, and S is the total toxicity (Marking, 1977, Howe et al., 1998).

For $S > 1$, the additive index is calculated as S (-1.0)+1.0 and for $S < 1$ the additive index is calculated as (1/S)-1.0 (Marking, 1977). These equations give the Corrected Sum-Additive Index (CSAI). CSAI <0 indicates a less than additive toxicity and those values that are >0 indicates a greater than additive toxicity. This index is used for this study rather than the Toxic Unit method because CSAI can assess additive toxicity of different ratios of chemicals within

the mixture (Marking 1977). The Corrected-Sum-Additive index was only calculated for the active ingredients because it was assumed toxicity for commercial formulations would increase due to the presence of surfactants, based upon published reports (Mann and Bidwell, 1999, 2001; Geisy et al., 2000; Coyner et al., 2000).

Split-plot ANOVA analyze were used to determine the effect of herbicides on growth rate due to date of laboratory testing, either as active ingredient or the commercial herbicide, and amount of stock solution added (S-Plus®, 2000 and SAS®, 2000). Photographs of the glass filters were taken with an Olympus dissecting microscope fitted with an Olympus single-reflex 35mm camera with an automatic shutter for a total magnification of 150X.

Graphs were constructed using Microsoft® Excel 97 and S-Plus® adding the trend line and trend line equation to determine R^2 values.

CHAPTER 4

RESULTS

Field Results

Chlorophyll concentration is used as an indicator of growth and measured as μg chlorophyll cm^{-2}. Chlorophyll species were measured as total chlorophyll, chlorophyll *a*, chlorophyll *b* and chlorophyll *c1+c2*. Table 8 shows the response of total chlorophyll against the amount of herbicide stock solution added to the periphytometer reservoir.

Table 8 shows there are no significant differences ($p<0.05$) between control values and treatment values with the exception of the lowest concentration of Glean using split-plot ANOVA. When compared to the control values, Figure 11 illustrates that each herbicide treatment has no adverse effect upon chlorophyll concentration.

Table 8. Mean total chlorophyll $\mu g/cm^{-2}$ (with error and 95% confidence interval) for periphyton exposed to three herbicides by the amount of stock added to the Matlock Periphytometer. Standard error and confidence intervals are values about the mean total chlorophyll.

Herbicide	Concentration of active ingredient mg L^{-1}	Amount of stock solution ml	Number of samples	Mean Total chlorophyll $\mu g/cm^{-2}$	Std error of mean	Upper 95% C.I.	Lower 95% C.I.
Glean	0.0	0	11	0.47	0.14	0.78	0.16
	7.8	1	10	0.63*	0.17	1.02	0.24
	15.6	2	9	0.32	0.07	0.49	0.16
	23.4	3	10	0.27	0.07	0.43	0.10
	31.2	4	11	0.31	0.08	0.49	0.13
	39.0	5	12	0.36	0.08	0.54	0.17
Roundup	0.0	0	14	0.18	0.03	0.24	0.12
	7.8	1	8	0.19	0.05	0.31	0.06
	15.6	2	10	0.17	0.03	0.22	0.11
	23.4	3	9	0.13	0.02	0.18	0.08
	31.2	4	8	0.19	0.05	0.31	0.08
	39.0	5	10	0.11	0.02	0.16	0.06
Aatrex	0.000	0	11	0.26	0.04	0.35	0.16
	0.003	1	10	0.24	0.04	0.33	0.14
	0.006	2	7	0.27	0.06	0.42	0.12
	0.009	3	9	0.24	0.05	0.35	0.13
	0.012	4	8	0.18	0.03	0.26	0.11
	0.015	5	9	0.20	0.03	0.28	0.13

* Value reflects significance at $p<0.05$ between controls and treatments.

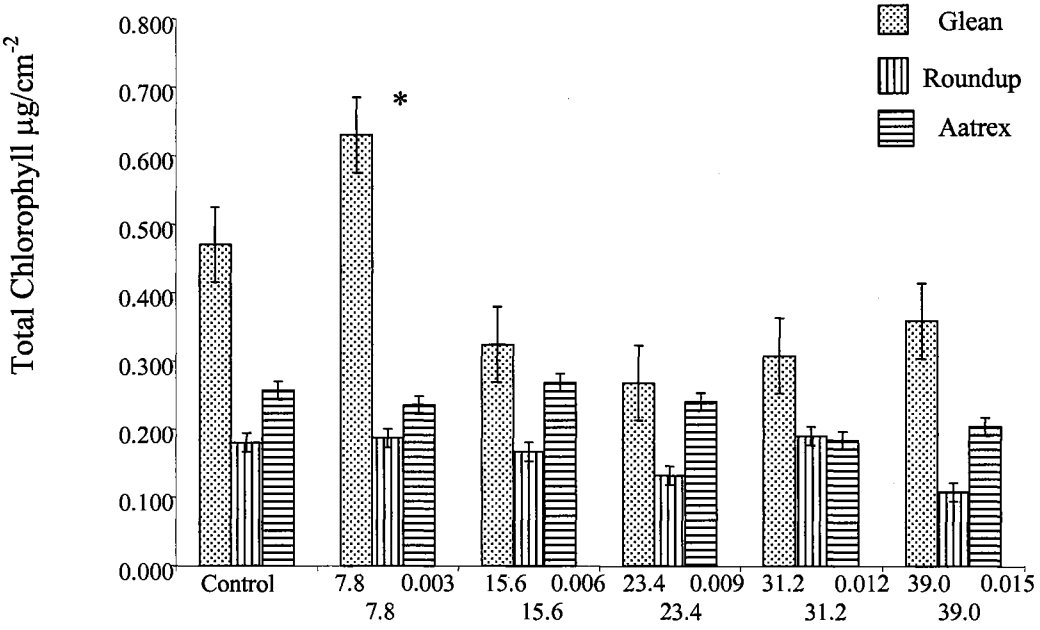

Figure 11. Total chlorophyll per cm^2 of growth surface. The comparison among the three tested herbicides and the concentration of stock solution (ml) added to the Matlock Periphytometer. Error bars represent the standard error about the mean.

Table 9 shows the Split-plot ANOVA analysis results for total chlorophyll. This table indicates that the herbicide*concentration interaction is significant at $p<0.05$. The interaction shown in Table 9 appears to be an artifact of test dates. Field-tests for each herbicide where conducted on two different days. The split-plot analysis looks for an interaction between test dates.

A test of slices from the split plot ANOVA analysis (Table 10) shows that the majority of this interaction is from Glean reflecting that Glean has a greater effect on total chlorophyll concentration than Roundup or Atrazine.

Table 9. Split plot ANOVA table for total chlorophyll from the Matlock Periphytometer. The α value set at 0.95 for all tables within this analysis.

Source	DF	Type III SS	Mean Square SS	F Value	Pr > F
Herb*Conc	10.0	0.435	0.043	2.440	0.010*
Date*Conc(Herb)	15.0	0.943	0.063	3.520	<.0001

* Indicates that this interaction has a significant result on the amount of total chlorophyll from the periphytometer filters.

Table 10. Split-plot ANOVA analysis, test of slices from the Tukey Multiple Comparison for total chlorophyll. The test compares each herbicide within each herbicide concentration.

Effect	Herb	DF	DF	F Value	Pr > F
Herb*Conc	Aatrex	5.0	16.0	0.12	0.986
Herb*Conc	Glean	5.0	14.4	2.66	0.067
Herb*Conc	Roundup	5.0	15.0	0.17	0.970

Chlorophyll *a* values (Table 11) are the highest fraction of the total chlorophyll measurements followed by chlorophyll *b*, and chlorophyll *c1+c2*. The presence of chlorophyll *b* and *c1+c2* indicate there are Chlorophyta and Chrysophyta in the stream. Comparing the herbicides shows no dose-response shown by total chlorophyll (Tables 8, 11-13). The exception is an increase in total chlorophyll 7.8 mgL^{-1} Glean.

Table 11. Mean chlorophyll *a* $\mu g/cm^{-2}$ (with error and 95% confidence interval) for periphyton exposed to three herbicides by the amount of stock added to the Matlock Periphytometer. Standard error and confidence intervals are values about the mean chlorophyll *a*.

Herbicide	Concentration of active ingredient mg L^{-1}	Amount of stock solution ml	Number of samples	Mean chlorophyll *a* $\mu g/cm^{-2}$	Std error of the mean	Upper 95% C.I.	Lower 95% C.I.
Glean	0.0	0	11	0.30	0.08	0.47	0.13
	7.8	1	10	0.31	0.08	0.50	0.11
	15.6	2	9	0.26	0.06	0.40	0.13
	23.4	3	10	0.22	0.06	0.35	0.09
	31.2	4	11	0.25	0.07	0.39	0.10
	39.0	5	12	0.28	0.06	0.41	0.14
Roundup	0.0	0	14	0.13	0.02	0.17	0.09
	7.8	1	8	0.17	0.05	0.29	0.05
	15.6	2	10	0.11	0.02	0.15	0.07
	23.4	3	9	0.10	0.02	0.14	0.07
	31.2	4	8	0.13	0.02	0.19	0.07
	39.0	5	10	0.08	0.02	0.12	0.04
Aatrex	0.000	0	11	0.20	0.04	0.29	0.11
	0.003	1	10	0.19	0.04	0.28	0.10
	0.006	2	7	0.22	0.05	0.35	0.09
	0.009	3	9	0.18	0.04	0.28	0.09
	0.012	4	8	0.14	0.03	0.21	0.06
	0.015	5	9	0.14	0.03	0.21	0.07

* Value reflects significance at $p<0.05$ between controls and treatments.

Table 11 shows there are no significant changes in the chlorophyll *a* biomass ($p<0.05$) when periphyton are exposed to any of the tested herbicides when compared to the controls. This trend is also seen in both chlorophyll *b* (Table 12) and chlorophyll *c* (Table 13) with the exception of the lowest concentration of Glean. It appears that these herbicides at these concentrations have little effect upon the periphyton growth as measured by chlorophyll species.

Table 12. Mean chlorophyll *b* $\mu g/cm^{-2}$ values (with error and 95% confidence interval about the mean) for periphyton exposed to three herbicides by the amount of stock added to the Matlock Periphytometer. Standard error and confidence intervals are values about the mean chlorophyll *b*.

Herbicide	Concentration of active ingredient mg L^{-1}	Amount of stock solution ml	Number of samples	Mean chlorophyll *b* $\mu g/cm^{-2}$	Std error of the mean	Upper 95% C.I.	Lower 95% C.I.
Glean	0.0	0	11	0.08	0.03	0.19	0.01
	7.8	1	10	0.10*	0.05	0.26	0.06
	15.6	2	9	0.04	0.01	0.07	0.01
	23.4	3	10	0.03	0.01	0.06	0.01
	31.2	4	11	0.04	0.01	0.07	0.01
	39.0	5	12	0.05	0.01	0.08	0.02
Roundup	0.0	0	14	0.03	0.01	0.04	0.02
	7.8	1	8	0.03	0.01	0.05	0.00
	15.6	2	10	0.03	0.01	0.04	0.01
	23.4	3	9	0.01	0.00	0.02	0.01
	31.2	4	8	0.03	0.01	0.06	0.00
	39.0	5	10	0.01	0.00	0.02	0.01
Aatrex	0.000	0	11	0.04	0.00	0.05	0.03
	0.003	1	10	0.03	0.01	0.04	0.02
	0.006	2	7	0.02	0.01	0.04	0.01
	0.009	3	9	0.03	0.00	0.04	0.02
	0.012	4	8	0.03	0.01	0.04	0.01
	0.015	5	9	0.04	0.00	0.05	0.03

* Value reflects significance at $p<0.05$ between controls and treatments.

However, at 7.8 mg L^{-1} of Glean increases in chlorophyll b and chlorophyll c concentration are significant by split-plot ANOVA and are indicative of both chlorophyte and chrysophyte, probably diatoms, population increases relative to cyanophytes. Because there are no apparent changes in chlorophyll *a* concentrations, the cyanophyte population appears not to be stimulated as the other groups are by Glean.

Table 13. Mean chlorophyll *c* $\mu g/cm^{-2}$ values (with error and 95% confidence interval) for periphyton exposed to three herbicides by the amount of stock added to the Matlock Periphytometer. Standard error and confidence intervals are values about the mean chlorophyll *c*.

Herbicide	Concentration of active ingredient $mg\ L^{-1}$	Amount of stock solution ml	Number of samples	Mean chlorophyll *c* $\mu g/cm^{-2}$	Std error of the mean	Upper 95% C.I.	Lower 95% C.I.
Glean	0.0	0	11	0.09	0.03	0.16	0.01
	7.8	1	10	0.11*	0.05	0.26	0.06
	15.6	2	9	0.04	0.01	0.04	0.01
	23.4	3	10	0.02	0.01	0.03	0.01
	31.2	4	11	0.02	0.01	0.03	0.01
	39.0	5	12	0.04	0.01	0.05	0.02
Roundup	0.0	0	14	0.03	0.01	0.04	0.02
	7.8	1	8	0.02	0.00	0.03	0.01
	15.6	2	10	0.03	0.01	0.05	0.01
	23.4	3	9	0.02	0.01	0.03	0.01
	31.2	4	8	0.03	0.02	0.07	-0.01
	39.0	5	10	0.01	0.00	0.02	0.01
Aatrex	0.000	0	11	0.02	0.00	0.03	0.01
	0.003	1	10	0.02	0.01	0.03	0.01
	0.006	2	7	0.03	0.01	0.04	0.01
	0.009	3	9	0.03	0.01	0.04	0.01
	0.012	4	8	0.02	0.00	0.03	0.01
	0.015	5	9	0.03	0.00	0.03	0.02

* Value reflects significance at $p<0.05$ between controls and treatments.

Chlorophyll Ratios

Tables 15-18 show the results of one-way ANOVA analysis of chlorophyll ratios. Because chlorophyll *a* is the most abundant pigment and is found in all algae and cyanobacteria, changes in the ratio of accessory pigments to chlorophyll *a* can indicate changes in dominant populations of periphyton (Hutchinson, 1967). There is an unexplained increase in chlorophyll ratios with the lowest

concentration of Glean. One explanation could be hormesis at this herbicide level. A second explanation is the size of the bandpass. Because the bandpass of the spectrophotometer used to measure the chlorophyll in this study was 8nm, the chlorophyll absorbance reading error could be higher than if a bandpass of >5 nm were used.

Other concentrations show slight declines from control values, which are non-significant and have no real dose-response (Table 14). The results indicate that Glean has a significant effect on both chlorophyll *b/a* and *c/a* ratios and Aatrex has a significant effect on chlorophyll *c/a* ratio at high concentrations. The majority of the effect from Glean can be accounted for at the 7.8 mg L^{-1} concentration.

Table 14. Mean chlorophyll *b/a* for field data at each herbicide concentration. The confidence intervals are not symmetrical around the mean because the use of ratios.

Herbicide	Herbicide Concentration mg L^{-1}	Chl *b/a* $\mu g/\mu g$	Lower 95% Confidence Level	Upper 95% Confidence Level
Glean	0.0	0.245	0.068	0.283
	7.8	0.342*	0.121	0.563
	15.6	0.137	0.080	0.195
	23.4	0.115	0.064	0.165
	31.2	0.117	0.059	0.175
	39.0	0.133	0.078	0.188
Roundup	0.0	0.245	0.140	0.266
	7.8	0.147	0.092	0.202
	15.6	0.286	0.088	0.485
	23.4	0.107	0.067	0.148
	31.2	0.245	0.078	0.411
	39.0	0.192	0.132	0.253
Aatrex	0.000	0.198	0.144	0.591
	0.003	0.131	0.037	0.474
	0.006	0.106	-0.111	0.536
	0.009	0.163	-0.034	0.972
	0.012	0.192	-0.068	0.931
	0.015	0.247	0.099	0.980

* Value reflects significance at $p<0.05$ between controls and treatments.

Table 15. Mean chlorophyll *c/a* for field data at each herbicide concentration. The confidence intervals are not symmetrical around the mean because the use of ratios.

Herbicide	Herbicide Concentration mg L^{-1}	Chl *c/a* $\mu g/\mu g$	Lower 95% Confidence Level	Upper 95% Confidence Level
Glean	0.0	0.176	0.107	0.318
	7.8	0.361*	0.135	0.586
	15.6	0.137	0.038	0.236
	23.4	0.095	0.011	0.178
	31.2	0.080	0.012	0.147
	39.0	0.136	0.117	0.155
Roundup	0.0	0.176	0.183	0.267
	7.8	0.139	0.054	0.224
	15.6	0.373	0.136	0.611
	23.4	0.195	0.093	0.298
	31.2	0.236	0.037	0.436
	39.0	0.192	0.120	0.264
Aatrex	0.000	0.082	0.043	0.118
	0.003	0.083	0.037	0.109
	0.006	0.112	0.085	0.132
	0.009	0.141	0.079	0.228
	0.012	0.164*	0.082	0.320
	0.015	0.171*	0.120	0.316

* Value reflects significance at $p<0.05$ between controls and treatments.

Table 16. One-way ANOVA analysis of chlorophyll *b/a* ratio to Roundup exposure.

Source	df	Sum of Squares	Mean Square	p value
Treatment	5	0.19	0.04	0.14
Residual	53	1.24	0.03	
Total	58	1.43		

Table 17. One-way ANOVA analysis of chlorophyll *c/a* ratio to Roundup exposure.

Source	df	Sum of Squares	Mean Square	p value
Treatment	5	0.3	0.06	0.13
Residual	53	1.77	0.03	
Total	58	2.06		

Tables 16 and 17 show the one-way ANOVA analysis of Roundup. At environmentally relevant concentrations of Roundup, there are no significant effects ($p<0.05$) on chlorophyll ratios of periphyton.

Tables 18 and 19 indicate the effect of Aatrex on chlorophyll ratios. While Aatrex has no significant effect on chlorophyll *b/a* ratios, there are significant effects on chlorophyll *c/a* ratios. This significance is primarily due to a reduction in chlorophyll *a* values and no change in chlorophyll *c* (Table 11, 13). There are no reductions chlorophyll *b* and *c* concentrations (Tables 12,13). Increases in chlorophyll *c/a* ratio are, therefore, due to the loss of chlorophyll *a* indication a decrease in cyanophytes.

Table 18. One-way ANOVA analysis of chlorophyll *b/a* ratio to Aatrex exposure.

Source	df	Sum of Squares	Mean Square	p value
Treatment	5	0.7	0.14	0.72
Residual	49	12.02	0.25	
Total	54	12.71		

Table 19. One-way ANOVA analysis of chlorophyll *c/a* ratio to Aatrex exposure.

Source	df	Sum of Squares	Mean Square	p value
Treatment	5	0.18	0.04	0.004*
Residual	49	0.44	0.01	
Total	54	0.63		

* Indicates a significant result $p<0.05$.

Table 20. Dunnett's multiple comparison of chlorophyll c/a ratio from Aatrex exposure. The comparison is between the control value and the treatment concentration mg L^{-1} value for each of the stock additions.

Concentration of Stock Solution of Atrazine μg L^{-1}	p value
Control vs 0.003	>0.05
Control vs 0.006	>0.05
Control vs 0.009	>0.05
Control vs 0.012	<0.05*
Control vs 0.015	<0.05*

* Value reflects significance at $p<0.05$ between controls and treatments.

This can also be seen when using Dunnett's multiple comparison (Table 20). The analysis indicates there are significant differences between the control values and the highest treatments. If compared with Tables 11 and 13, the reductions causing the significant values are reductions in chlorophyll *a*. There are no specific p-value calculations with the Dunnett's comparison, so the actual value for p is not known.

Table 21. One-way ANOVA analysis of chlorophyll *b/a* ratio to Glean exposure.

Source	df	Sum of Squares	Mean Square	p value
Treatment	5	0.39	0.08	0.01*
Residual	57	1.36	0.02	
Total	62	1.74		

* Value reflects significance at $p<0.05$ between controls and treatments.

Table 22. One-way ANOVA analysis of chlorophyll *c/a* ratio to Glean exposure.

Source	df	Sum of Squares	Mean Square	p value
Treatment	5	0.55	0.11	0.003*
Residual	57	1.51	0.3	
Total	62	2.06		

* Value reflects significance at $p<0.05$ between controls and treatments.

Tables 21 and 22 support Table 10 indicating that Glean has a significant impact upon the chlorophyll ratios of algae. One-way ANOVA analysis shows at low concnentrations Glean has a stimulatory effect upon chlorophyll *b* and *c* (Tables 12 and 13). This stimulkatroy effect suggests growth of eukaryotic periphyton relative to the cyanobacteria.

A negative control utilizing a 1-% $CuSO_4$ was used to determine if complete inhibition of growth of periphyton could be obtained. As Figure 26 (Appendix A) shows, there is little growth of periphyton on the filter indicating that with a large dose of a toxic material, periphyton growth can be inhibited.

Active Ingredient Study

In an effort to determine if the herbicides have impact upon periphyton, the active ingredient of each herbicide was used to study the differences in growth rates and chlorophyll concentration responses from *P. oedogonia*. *P. oedogonia* is a filamentous algae that grows in slow moving lotic and lentic environments (O'Neal and Lembi, 1983) and was added to the field experiment flume.

Also, experiments were performed to compare the growth rates to commercial formulation and surfactants alone. With these studies, I attempted to determine if *P. oedogonia* could be used as a suitable indicator organism. Wendt-Rasch et al. (2003a) suggests that bottle tests can overestimate the toxicity of herbicides compared to field experiments and that the results need to be evaluated using both methods.

Table 23. One Way Analysis of Variance of active ingredient formulation of herbicide effect on growth rate of *P. oedogonia*

Treatment	Mean Square	F Value	P Value
Atrazine	0.0052	15.2	0.030*
Chlorsulfuron	1673.39	0.91	0.024*
Glyphosate	512651.8	9.55	0.091

* Value reflects significance at $p<0.05$ between controls and treatments.

Table 23 shows the results of a one-way ANOVA to determine if there is a significant effect of herbicides on growth rate. There was significance at the $p<0.05$ level for atrazine and chlorsulfuron but not for glyphosate. Table 24 shows that cellular chlorophyll *a* content remained constant for all of the treatments, indicating that the herbicides, at low Photon Flux Density (PFD) levels, did not interfere with chlorophyll synthesis or stability, independent of growth (O'Neal and Lembi, 1983). This suggest these herbicides do not significantly affect light-harvesting ability of *P. oedogonia* and that chlorophyll *a* concentration may be appropriate to measure growth rate. However, the difference in fresh weight of algae per unit of time maybe a better indicator of growth rates for this study.

Table 24. Effects of active ingredients glyphosate, chlorsulfuron, and atrazine on growth rate, μg Chlorophyll *a*/mg Fresh weight of *Pithophora oedogonia*. The standard error is the standard around the mean of the data.

Treatment	Conc. mg/L	μg Chlorophyll *a*/mg fresh weight	Growth Rate mg fresh weight/day	STD Error of the mean	Upper 95% C.I.	Lower 95% C.I.
Glyphosate	Control	0.979	0.357	0.012	0.307	0.115
	100	0.680	0.128*	0.019	0.038	-0.113
	500	0.961	0.035*	0.050	0.234	-0.245
	1000	0.967	0.000*	0.036	0.000	0.000
Chlorsulfuron	Control	0.847	0.311	0.012	0.349	0.274
	0.50	0.845	0.295	0.019	0.357	0.234
	5.0	0.859	0.235	0.050	0.396	0.075
	50.0	0.836	0.076*	0.036	0.189	-0.037
Atrazine	Control	0.869	0.312	0.048	0.464	0.159
	0.025	0.973	0.264	0.051	0.430	0.104
	0.050	0.852	0.195*	0.068	0.413	-0.023
	0.075	0.899	0.032*	0.118	0.441	-0.311
	0.10	0.788	0.090*	0.037	0.196	-0.036

* Value reflects significance at $p<0.05$ between controls and treatments.

Table 25 shows EC_{50} values calculated for each active ingredient of commercially available herbicide. These values fall within the range of published data for unicellular algae, and indicate that *P. oedogonia* can be a useful test organism for toxicology studies. The EC_{50} value for atrazine was extrapolated using non-linear regression and was used only for the calculation for the Corrected Sum-Additive index.

Although the calculated value for atrazine is an EC_{25} value, it does provide information. At 38 μg L^{-1} atrazine has an inhibitory effect on the growth rate of *P. oedogonia* and can be used to make toxicity comparisons with published studies (Linda and Girlish, 2000).

Table 25. 7-day EC_{25} and EC_{50} values with confidence intervals for the active ingredients found in Glean, Aatrex and Roundup. (Nystrom et. al., 1999, Solomon et al. 1996, Giesy et al. 2000)

Herbicide	EC_{50}	EC_{25}	Confidence Interval 95% for EC_{50} (EC_{25})	Published EC_{50} values for the herbicides
Chlorsulfuron (mg L^{-1})	28.0	6.0	4.8-44.00	0.016-275.5
Atrazine (μg L^{-1})	60.0#	38.0	15.6-54.5	21.0-1000.0
Glyphosate (mg L^{-1})	67.0	42.0	43.00-80.3	8.0-189.0

#The EC_{50} value for atrazine was calculated using (Graphed) non-linear regression procedure to use only for the calculation of the CSAI.

Active Ingredient-Mixture Study

Algae exposed to mixtures of active ingredients of Aatrex, Glean, and Roundup, showed a decreased growth rate (Table 26). The reduction in growth rate for *P. oedogonia* is more pronounced when exposed to herbicide mixtures as compared to the individual active ingredient. Table 26 shows the reduction in growth rates of both the active ingredient mixtures and the growth rate at EC_{50} value for the individual active ingredient. Table 26 also displays the Corrected-Sum-Additive Index value as proposed by Marking (1977) for each of the active ingredient mixtures.

Table 26. Effects of tandem active ingredients on growth rate, μg chlorophyll/mg fresh weight of *Pithophora oedogonia* including the Corrective Sum-Additive Index (CSAI). Included are standard errors of the mean.

Treatment	Conc. mg/L at the EC_{50} level	μg Chlorophyll *a*/mg fresh weight with std error of mean	Growth Rate mg fresh weight/day with std error of mean	CSAI	
Control	0.00	1.02 ± 0.03	0.31 ± 0.02		
Atrazine + Chlorsulfuron	0.06/28.0	1.28 ± 0.04	0.08 ± 0.04	-0.19	Antagonistic
Atrazine + Glyphosate	0.06/67.0	1.29 ± 0.06	0.05 ± 0.03	0.28	Additive
Chlorsulfuron + Glyphosate	28.0/67.0	1.02 ± 0.05	0.02 ± 0.02	0.64	Synergistic

Chemicals in mixture can have an additive, antagonistic, or synergistic effect on organisms being tested (Marking, 1977). The mixture of atrazine and chlorsulfuron (A/C) had CSAI value of –0.19 indicating a less-than-additive or antagonistic effect (Table 26). The average growth rate for the A/C mixture is 0.08-mg fresh weight per day (Table 26). Of the three mixtures, this value is the highest. The atrazine/glyphosate mixture had a CSAI value of 0.28 indicating an additive effect of these herbicides. This mixture had an average growth rate of 0.05-mg fresh weight/day, which was the second largest rate. The mixture of chlorsulfuron and glyphosate had the largest CSAI value of 0.64 (Table 26). The average growth rate for this mixture was 0.02-mg fresh weight/day. This value indicates that there is a greater-than-additive or synergistic effect of these herbicides on algal growth. Although the calculation of the CSAI determined that there is a less-than-additive effect of the chlorsulfuron/atrazine mixture, the mixture has a greater inhibitory effect than either individual active ingredient.

Commercial Formulation Study

As a follow-up study to the active ingredient study and the field study, the effects of commercially available herbicides were tested using *P. oedogonia*. Growth rates significant decreased when exposed to the commercial formulations of atrazine, glyphosate, and chlorsulfuron as well as the surfactant X-77 alone (Table 27). This reduction is similar to the commercial herbicides with the exception of Roundup. Roundup showed significant results only at the higher concentrations. The exact concentrations of X-77 in commercial formulations are not known, but the amounts of surfactant added to the bottles are similar to the tank mixtures recommended by the manufacturer. X-77 is a surfactant that is recommended for use with glyphosate in the form of the herbicide Rodeo for aquatic applications.

Table 27. Growth rate of *Pithophora oedogonia* as affected by commercial herbicides and surfactant X-77 in mg fresh weight/day. Standard errors of the mean of the data are given in parenthesis. Concentrations of herbicides can be found in Table 6.

Treatment	Glean	Aatrex	Roundup	X-77 Surfactant
Amount of stock solution added to culture flask ml.	--mg fresh weight /day--			
0	0.23 (0.02)	0.21 (0.03)	0.25 (0.03)	0.23 (0.03)
1	0.13* (0.04)	0.03* (0.03)	0.24 (0.03)	0.10* (0.05)
2	0.04* (0.02)	0.01* (0.01)	0.23 (0.03)	0.04* (0.06)
3	0.05* (0.01)	0.01* (0.004)	0.13* (0.05)	0.02* (0.06)
4	0.05* (0.02)	0.01* (0.004)	0.13* (0.04)	0.02* (0.03)
5	0.02* (0.004)	0.00* (0.002)	0.12* (0.04)	0.01* (0.03)

* Value reflects significance at $p<0.05$ between controls and treatments.

Table 28 compares the Least Squares Means estimates of growth rates of all commercial herbicides. The Least Squares Means are mean values that have been corrected for the imbalances in other variables (Steel et al., 1997).

Table 28. Least Squares Means Multiple Comparisons (Tukey test) of values within each herbicide using growth rate as the dependent variable. The estimate is the Least Square mean values found in Appendix B (page 132). The same letter after each value represents similar comparisons. A different letter represents different comparisons.

Treatment	Conc. mg L^{-1}	Est.	Group
Aatrex			
0	0	0.21	A
1	0.003	0.001	B
2	0.006	0.004	B
3	0.009	0.05	B
4	0.012	0.03	B
5	0.015	0.03	B
Glean			
0	0.0	0.30	A
1	7.8	0.09	B
2	15.6	0.03	B
3	23.4	-0.02	B
4	31.2	0.06	B
5	39.0	-0.02	B
Roundup			
0	0.0	0.26	A
1	7.8	0.30	A
2	15.6	0.23	AB
3	23.4	0.10	B
4	31.2	0.08	B
5	39.0	0.14	AB

These values can also be considered the best linear-unbiased estimates of the marginal means for that population (Steel et al., 1997). These values are all corrected for growth as mg fresh weight algae per day.

All concentrations of Glean and Aatrex have values significantly lower that the controls, but not significantly different from other concentrations of herbicides.

Roundup® demonstrates a BI-modal dose-response using Toukai's Multiple Comparison test, where treatments for 23 mg L^{-1} and 31 mg L^{-1} differ significantly from the control.

Table 29 shows the EC_{50} and EC_{25} values for each of the nominal commercial herbicide concentrations for recommended (label) application rates. The calculated values are within the ranges of published values (Nystrom et. al., 1999, Solomon et al. 1996, Giesy et al. 2000).

Table 29. EC_{50} and EC_{25} values (7-day) for Glean, Aatrex, and Roundup using growth rate as the dependent variable (Nystrom et. al., 1999, Solomon et al. 1996, Giesy et al. 2000)

Herbicide	EC_{50}	EC_{25}	Confidence Interval 95% for EC_{50}	Published EC_{50} values for each herbicide
Glean mg L^{-1}	6.0	3.2	4.75-11.27	0.016-275.5
Aatrex mg L_{-1}	0.02	0.009	0.016-0.027	0.021-1.00
Roundup mg L^{-1}	N/A	17.6	12.66-26.3	8.00-189.00

The EC_{50} value for Roundup could not be calculated because the concentration range for this study was not sufficiently wide but were sufficient to calculate an EC_{25} value.

The Split-plot ANOVA analyses (Table 30,31) for herbicides tested, at each concentration, indicate that all variables were significant for growth rate of *P. oedogonia*.

Table 30. Split-Plot ANOVA analysis of the effects commercial herbicide using growth rate as the dependent variable.

Source	df	Type III SS	Mean Square	F Value	Pr > F
Conch	5	0.97	0.19	19.69	<.0001
Herb*Conc	10	0.35	0.04	3.55	0.00*

To determine if growth rate is the only variable affected by herbicide concentration, the chlorophyll content was analyzed. Table 31 shows the Type III sum of squares result from using chlorophyll content as the dependent factor. The concentration of herbicide and the concentration*herbicide interaction are non-significant. These herbicides do not appear to affect algal chlorophyll *a* content.

Table 31. Split Plot ANOVA Table with μg Chlorophyll *a* /mg Fresh weight as Dependent Factor

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Conch	5	1.542	0.308	1.74	0.1294
Herb*Conc	10	2.015	0.202	1.14	0.3386

CHAPTER 5

DISCUSSION

Use of Matlock Periphytometer to deliver herbicides to periphyton communities

The Matlock Periphytometer is a passive diffusion periphytometer used previously to study trophic status of streams in Northeastern Oklahoma (Matlock et al. 1998, 1999A, 1999B). Here I investigated the suitability of the Matlock Periphytometer to simulate the addition of herbicides into lotic environment.

Control biomass, as chlorophyll *a*, was compared to previously published data (Table 32) for treatments having Matlock Periphytometer nutrient concentrations means closest to 20 mg L^{-1} phosphorous and 20 mg L^{-1} nitrogen and having an individual chlorophyll *a* values ranging from 0.98-5.87 μg chlorophyll *a* cm^{-2} (Matlock et al., 1999b; Keyworth, 2000). Chlorophyll *a* values from the control range from 0.16 μg chlorophyll *a* cm^{-2} for Roundup to 0.30 μg chlorophyll *a* cm^{-2} for Glean, indicating that chlorophyll measured in the flume can be used as an indicator of periphyton community growth.

The comparisons between this study and previously published studies indicate that chlorophyll *a* values from this study are slightly below published range of

0.28 to 0.55 μg chlorophyll cm $^{-2}$ for reported fall chlorophyll values (Matlock et al., 1998, 1999a, 1999b).

Table 32. Comparison of chlorophyll *a* values from Battle Creek, Peashooter Creek, Tyner Creek, and this study. Values for this study were determined by mean chlorophyll *a* values from control periphytometers. (Adapted from Matlock et al. 1998, 1999A, 1999B, Matlock 1999, Keyworth 2000)

Source	Season	Biomass as μg Total Chlorophyll *a* cm $^{-2}$	Reference
Battle Creek*	Spring	1.05	Matlock et al., 1999a
	Fall	0.28	
	Spring	1.07	Matlock, 1999
Peacheater Creek*	Spring	0.51	Matlock et al., 1999a
	Fall	0.28	
	Spring	0.50	Matlock, 1999
Tyner Creek*	Spring	0.21	Matlock, 1999a
	Fall	0.55	
	Spring	0.21	Matlock, 1999
This Study	Summer/ Fall	0.21	

*Matlock et al. (1999b) and Keyworth (2000) reported chlorophyll levels 0.98-5.87 and 1.0-9.6 μg chlorophyll cm $^{-2}$ respectively in Battle, Peacheater, and Tyner Creeks but did not indicate seasons or concentration levels of nutrients.

Community analysis after exposure to herbicides

To determine change in community structure, chlorophyll *a, b, c1+c2* and total chlorophyll were measured and compared. This is a new use for the Matlock Periphytometer, which was designed to measure trophic status of streams (Matlock et al., 1998, 1999a, 1999b). These measurements can show changes in relative abundance of the periphyton groups Chlorophyceae, Chrysophyceae (especially diatoms), and Cyanophyceae, the three most likely occurring divisions of

periphyton (Wilhm and Long, 1969; Ledger and Hildrew, 1998; Guasch et al., 2002). Chlorophyceae contain chlorophyll *a* and chlorophyll *b*, but no chlorophyll *c*, Chrysophyceae contain chlorophyll *a* and chlorophyll c_1+c_2 but no chlorophyll *b* and Cyanophyceae contain chlorophyll *a* but any *b* or *c*. By measuring the amounts of the different pigments, approximate community compositions can be compared (Nobel, 1999). The ratio for chlorophyll *a* to chlorophyll *b* and chlorophyll *a* to chlorophyll c_1+c_2 should be approximately 3:1 (Nobel, 1999). In a mixed community with both eukaryotic and prokaryotic members the ratio should ideally be 6:1:1 or higher, chlorophyll *a* to *b* to c_1+c_2 (Nobel, 1999). Analyzing all the chlorophyll species reveals more community information. The relative proportion of chlorophyll species can give some indication of dominant periphyton on a growth substrate (Ledger and Hildrew, 1998). Chlorophyll analysis of communities is faster than taxonomic work to identify the organisms present. Most diatom species require identification by an expert, which can delay the study (Barbour et al., 1999).

However, the measured chlorophyll ratios did not approach that ideal value. An explanation could be the spectrophotometer had a wider than ideal bandpass (8 nm) and thus some chlorophyll values may have been missed in the measuring of the chlorophyll species.

Environmentally realistic concentrations (concentrations of herbicides that can be expected to be found in streams as the result of normal agricultural practices) of Roundup, Glean, and Aatrex (Tables 9, 33) had no significant effect, by split-plot ANOVA, on the biomass accumulation of periphyton, measured as total

chlorophyll (μg chlorophyll cm^{-2}) (Guasch et al, 2002). Because there were no significant differences (at $p< 0.05$) between the controls and treatment groups, the null hypothesis, that there will be no effect on chlorophyll concentrations, could not be rejected. Table 33 presents concentrations from the literature and from the experimental conditions presented here.

Table 33. Herbicide concentrations found from overspray conditions as tested for from water samples from lotic environments and test concentrations used in this study.

Herbicide	Experimentally used concentrations	Environmentally realistic concentrations obtained from the literature.	Reference
Atrazine	0.0-20 μg L^{-1}	0.2-1000 μg L^{-1}	Nelson et. al (1999)
Chlorsulfuron	0.0-39.0 mg L^{-1}	3.0-20 mg L^{-1}	Peterson (2001)
Glyphosate	0.0-39.0 mg L^{-1}	0.06-21.2 mg L^{-1}	Goldsborough and Brown (1987)

The results in Figure 11(page 63) show an absence of dose-response effect on the amount of total chlorophyll from the periphytometer filters. Figure 11 also suggested a positive effect of Glean on periphyton, with an increased chlorophyll *b* and *c* concentrations at the lowest concentration. This increase may be the result of Glean stimulating the periphyton on the filter, described as possible hormesis. Hormesis is the stimulation of organism performance occurring at low exposure levels where exposure at higher levels is inhibitory (Forbes, 2000). The increase could also be due to a reduction in competition from other periphyton species.

Evaluating the individual chlorophyll species (Tables 11-13), Glean at the lowest concentrations indicated a significant increase in chlorophyll *b* and *c*

compared to the control groups ($p<0.05$). All other treatments were non-significant ($p<0.05$) regardless of the herbicide type or herbicide concentration. These data suggest chlorophytes and chrysophytes (diatoms) are favored at low exposure to Glean (Guasch et al., 2002), and at higher concentrations of Glean, all groups of periphyton maybe affected equally. Suggesting at low concentrations of Glean that chlorophytes and diatoms to become dominant relative to cyanobacteria (Ledger and Hildrew, 1998).

The absence of a dose-response when periphyton are exposed to these herbicides does not preclude effects higher in the food web (Nelson et al., 1999). Graymore et al. (2001) reports that the cladoceran, *Simocephalus serrulatus*, a zooplankton, decreased in abundance when their food supply was exposed to an environmentally relevant concentration of atrazine. The resultant loss of non-predatory macroinvertebrate diversity upon exposure to atrazine coincides with declines in periphyton and detritus communities (Gruessner and Watzin, 1996). Further, deNoyelles et al. (1982) determined that shifts in zooplankton dominance occur as atrazine exposure shifts periphyton communities in ponds. In these studies chlorophyll *a* levels were unaffected (deNoyelles et al., 1982; Graymore et al., 2001)

Chlorophyll Ratios

The tri-chromatic equation (Jeffrey and Humphrey, 1995) was used to determine the community composition. The equation uses the optical density of each chlorophyll species in relation to each other to determine the chlorophyll

content. The bandpass of the spectrophotometer is an important factor. The spectrophotometer used for this study had a bandpass of 8 nm. Because of the bandpass, the spectrophotometer may be reading absorbance of the chlorophyll species at lower than actual concentrations but the effect would be small. The data presented here are a precise reflection of the community structure in this lotic system because of the relative differences between these values.

These data show that there is a great deal of diversity and complexity in periphyton community response to the three selected herbicides suggesting that herbicide exposure may change community structure (Guasch et al, 2002). To determine the change in the composition of periphyton community, ratios of chlorophyll *b* to *a* and c_1+c_2 to *a* were compared (Figures 12, 14, 16). For example, a higher chlorophyll *c/a* ratio suggests a shift toward diatoms within the community (Ledger and Hildrew, 1998). Likewise a higher chlorophyll *b/a* suggests a shift in community toward green algae. Chlorophyll *c/a* and *b/a* ratios that appear equal to each other suggest a reduction in green algae and diatoms, in proportion to the presence of cyanobacteria (Ledger and Hildrew, 1998).

Using a One-Way Analysis of Variance ($p<0.05$), Figure 12 illustrates there is no clear dose-response relationship of chlorophyll ratios to Roundup.

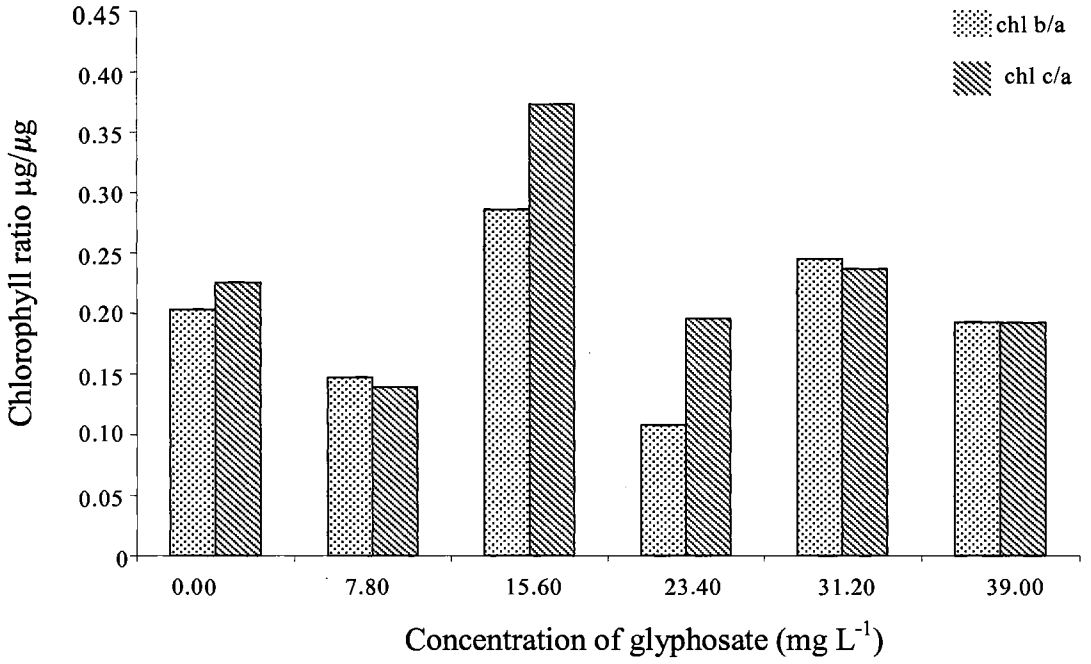

Figure 12. Chlorophyll ratios for periphyton exposed to Roundup using the Matlock Periphytometer.

At 15.6 mg L^{-1} of Roundup, there appears to be an increase in the ratio of chlorophyll *b* and *c* relative to chlorophyll *a* suggests the greens and diatoms had increased relative to the cyanophytes, but this increase is reversed at 23 mg L^{-1}. However, for Roundup, these results are not significant ($p<0.05$). This lack of inhibition may be because of the short half-life of glyphosate (Geisy et al., 1996). Table 8 showed there was no significant change in total chlorophyll concentration with herbicide addition from the control to the highest concentration.

The photograph, Figure 13, shows typical filamentous growth on the glass filter exposed to Roundup. The statistically non-significant ratio ($p<0.05$) seen in Figure 12 between chlorophyll *b/a* and *c/a* would suggest a reduction in the cyanophyte population relative to the chlorophyte and chrysophyte populations.

This is contrary to Tsui and Chu (2003) who determined that chrysophytes are 7-10 times more sensitive to Roundup.

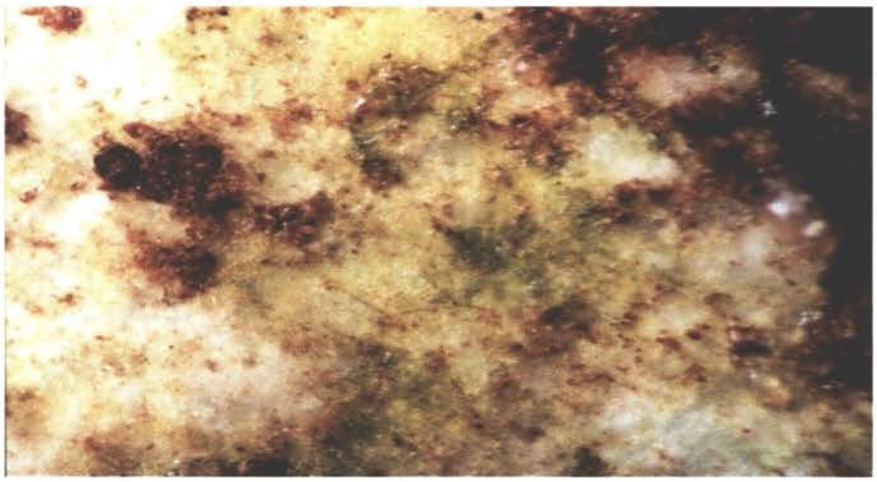

Figure 13. Photograph of the glass filter from Roundup (23.40 mg L^{-1}) added to the Matlock Periphytometer. Periphyton on the filter has not been identified. (Total magnification 150X)

Aatrex reduces chlorophyll *b/a* and *c/a* ratios at low herbicide concentrations and increase ratios at higher herbicide concentrations (Figure 14). Chlorophyll *c/a* but not *b/a* ratios increased significantly at the higher herbicide concentrations (Tables 18 and 19). The increase ratio at higher concentrations is due to an increase in chlorophyll *c* and a decrease in chlorophyll *a*.

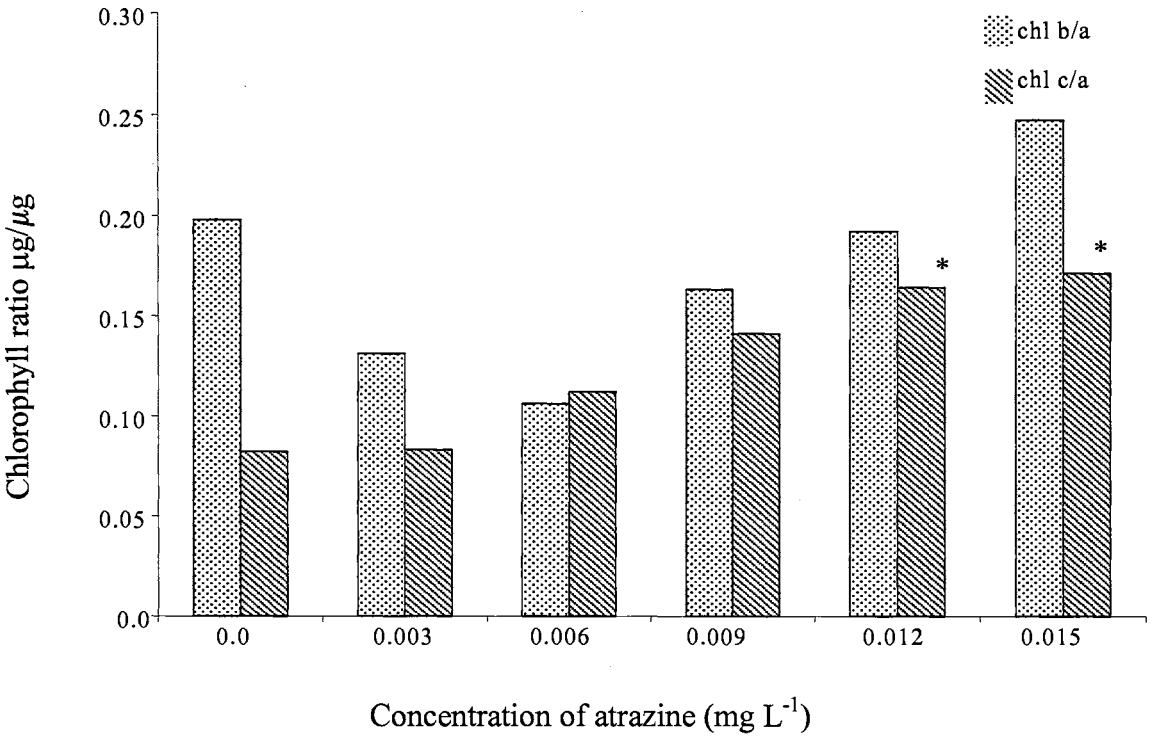

Figure 14. Chlorophyll ratios for periphyton exposed to Aatrex using the Matlock Periphytometer.

Because the ratios of chlorophyll *b/a* and *c/a* appear to be constant at the lower concentrations of Aatrex, the results suggest a decreasing cyanophyte population relative to chlorophyte and chrysophyte populations (Guasch et al., 2002). At higher concentrations of Aatrex, there is a significant increase ($p<0.05$) in chlorophyll c/a ratio (Table 15) indicating that cyanophytes may are affected more than chlorophytes and chrysophyte. This suggests that Aatrex has a greater inhibitory effect upon prokaryotic periphyton rather than eukaryotic periphyton. The suggestion that prokaryotic organisms rather than eukaryotic periphyton are affected greater may lie in the lack of cytoplasmic organelles such as perioxisomes (Nobel, 1999). Perioxisomes contain enzymes that have the ability to breakdown foreign chemicals that are introduced into the cell.

This suggestion that prokaryotes are impacted, and is contrary to published reports that indicate a reduction in all species of periphyton. For example, Nelson et al. (1999) reported diatoms (chrysophyte) exposed to 83 $\mu g\ L^{-1}$ of atrazine for 7 days had reduced growth rates.

Table 20 (page 71) shows the results of Dunnett's multiple comparison of a One Way ANOVA, comparing control vs each concentration of Aatrex for chlorophyll *c/a*. The higher concentrations are significantly ($p<0.05$) different from the control. Yet Tables 8, 11-13 indicated no significant differences from the control for each individual chlorophyll species and for total chlorophyll. This is similar to the results seen with Roundup. The explanation for the difference in chlorophyll ratio and individual chlorophyll species can be a shift in dominance from cyanophytes to chrysophytes (Guasch et al. 1997, Guasch et al., 1998). Solomon et al. (1996) showed a similar effect with lake phytoplankton.

Pratt et al. (1988) reported that a stimulation of growth of microbial communities, including an increase in chlorophyll *a* concentration, occurs when they are exposed to low concentrations of atrazine. However, Solomon et al. (1996) determined that low concentrations of atrazine did not inhibit growth of periphyton in artificial streams, and atrazine has no effect on the recruitment and survival of periphyton. In contrast, Nelson et al. (1999) demonstrated that diatom populations were reduced when exposed to 83 $\mu g\ L^{-1}$ atrazine for 12 days.

Figure 15 is a photograph taken from a Matlock Periphytometer exposed to 0.006 $mg\ L^{-1}$ Aatrex. There are few colonies of periphyton on this or other periphytometers from Aatrex exposure.

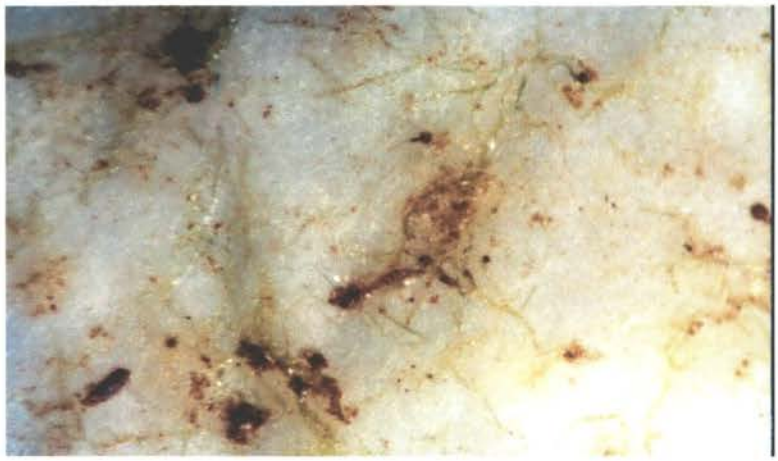

Figure 15. Photograph of the glass filter from a Matlock Periphytometer exposed to 0.006 mg L^{-1} Aatrex. (Total Magnification 150X)

Figure 16 shows the changes in chlorophyll ratio for periphyton exposed to Glean. Glean seems to affect eukaryotic algae and not prokaryotic algae. At the lowest concentrations of Glean, there is an increase in growth of chlorophytes and chyrsophytes relative to cyanophytes. Chlorophyll *a* values (Table 11) remain constant at all concentrations of Glean. However, chlorophyll *b* and *c* values (Tables 12 and 13) increase at the lowest concentration of Glean (7.8 mg L^{-1}). At higher concentrations of Glean, the number of chlorophytes and chrysophytes decline relative to cyanophytes. A stimulatory effect of Glean has not been suggested previously in the literature (Fletcher et al., 1996, Strek, 1999 a, b).

This increase at lower Glean concentrations may be attributed to the phenomenon of hormesis. Hormesis is a stimulatory effect that increases the physiological process being studied to 30-60% above control values (Chapman, 2002). Hormesis is thought to be the overcompensation of an organism to alterations in homeostasis (Chapman, 2002). Chapman (2002) suggests that hormesis is not only a toxicological process but can also be an ecological process.

However, some intermediate response to a disturbance involving both natural and physical stressors (Chapman, 2002). The hormesis dose-response curve shows a stimulatory response at low concentrations and toxic response at high concentrations.

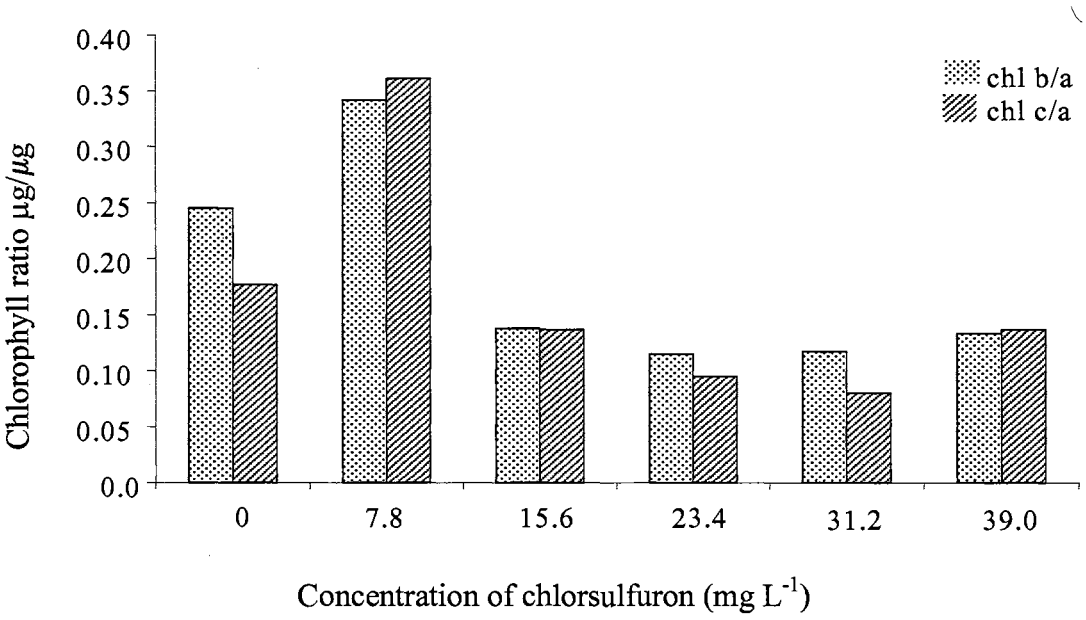

Figure 16. Chlorophyll ratios for periphyton exposed to Glean using the Matlock Periphytometer.

At low levels of stress, a few species may become competitively dominant. At higher levels of stress those few species may become less competitive, and there maybe an increase in diversity (Chapman, 2002).

Figure 17 illustrates periphyton that was present on the growth surface from exposure to Glean. On this photograph, chrysophytes can plainly be seen. Seeming to indicate that chrysophytes are better able to tolerate Glean. The chlorophyll ratios (Figure 17) however indicate that there is an equal proportion of chrysophytes to chlorophytes within the total community.

Figure 17. Photograph of a glass filter from a Matlock Periphytometer exposed to 31.0 mg L^{-1} Glean showing a grouping of unidentified pennate diatoms. (Total Magnification 150X).

Bottle Test for the effects of commercial formulation and active ingredients upon *Pithophora oedogonia*

Following the field experiments, where herbicides failed to elicit a clear dose-response in a mixed community with varying conditions, I conducted bottle tests using *P. oedogonia* as a test organism. *P. oedogonia* is a filamentous chlorophyte found in low velocity streams and in small lakes and ponds. *P. oedogonia* was exposed to herbicide concentrations typical of tank mixtures and used for the periphytometer study.

The objective of this study was to determine the effects of the commercial formulations and the active ingredients of Roundup (glyphosate), Glean (chlorsulfuron), and Aatrex (atrazine) on the growth and cellular chlorophyll *a* concentrations of *P. oedogonia*. The results indicated that growth rates for *P. oedogonia* were significantly reduced, but that the cellular chlorophyll *a* was not

affected. As found in this study, Guasch et al. (2003) suggested that atrazine toxicity does not affect chlorophyll content. Periphyton exposure to atrazine for 36 days demonstrated an increase in chlorophyll content (Guasch et al., 1997). Nelson et al. (1999) indicated that colonies of *Craticula cuspidata* (Chrysophyta) the concentration of chlorophyll *a* did not decrease when chronically exposed to 1 $\mu g\ L^{-1}$ atrazine. When exposed to short-term (acute) concentrations of atrazine (0-3,250 $\mu g\ L^{-1}$), growth was inhibited but chlorophyll as µg/mg of fresh weight, concentration remained constant or increased (Nelson et al., 1999).

Comparing the data from both commercial formulations and active ingredients, commercial formulations have a greater effect upon growth rates. One reason for the difference may be the presence of surfactants. This is contrary to Saenz et al. (1997) who indicated that both the technical (active ingredient) and commercial forms of glyphosate could reduce growth rates of algae, but that only technical formulation produced significant reductions. However, Saenz et al. (1997) also indicated that extending the time of exposure from 48 hours to 96 hours increased the toxicity of the commercial formulation of glyphosate. Bolognesi et al. (1997) suggested commercial formulations are more toxic than the active ingredient alone.

Mann and Bidwell (2001) reported that amphibians died when exposed to Roundup in low concentrations. They also determined that the surfactants used in Roundup had the same dose response effects as the active ingredient. Geisy et al. (2000) also indicated that the EC_{50} values (96 hour) for Roundup are lower than glyphosate alone, however, indicated that this difference is not significant. Tsui

and Chu (2003) determined that the surfactant in Roundup is toxic to marine copepods but not to algae.

The non-ionic form of surfactants should have an increased toxicity because of nonspecific membrane disruption (Leaper and Holloway, 2000). Paveglio et al. (1996) describes the surfactant X-77 as being a non-ionic nonylphenol polyethoxylate. The literature, with respect as to the exact effect surfactants have on algae, is divided (Paveglio et al., 1996, Simenstad et al., 1996, Bolognesi et al., 1997, Leaper and Holloway, 2000, Hense et al., 2003, Tsui and Chu, 2003,).

The results presented here agree with Bolognesi et al. (1997) that the commercial forms of these herbicides have the greater adverse affect upon algae, at least in bottle tests.

P. oedogonia's response to Aatrex (Figures 22-23) showed the most pronounced reduction in growth rates with all concentrations of herbicide having a significant effect. Nelson et al. (1999) reports that at high concentrations of atrazine (>83 $\mu g\ L^{-1}$) growth rates cannot be calculated because of direct inhibition to the plastoquinone complex. The effect of atrazine is more pronounced when mixed with a surfactant. In commercial formulations (Aatrex), the growth rates of *P. oedogonia* are significantly reduced. When exposed to the active ingredient alone, only the highest concentrations of atrazine produce a significant result, which suggests adding a surfactant increases toxicity (Geisy et al., 2000). Fairchild et al. (1998) demonstrated that 96 hr EC_{50} values for 4 green algae ranged from 94-176 $\mu g\ L^{-1}$ when exposed to atrazine. Fairchild et al (1997) also determined that atrazine has a 96 hr EC_{50} value for *Selenastrum*

capricornutum to be 234 $\mu g\ L^{-1}$. The calculated EC_{50} value for atrazine in this study was 60 $\mu g\ L^{-1}$.

Active ingredients and commercial formulations of Glean and Roundup also produced exponential dose reduction in growth rates, again with the commercial herbicide having a greater reduction. For example, chlorsulfuron reduced growth of *P. oedogonia* to 100-mg fresh weight day^{-1} at 50-mg L^{-1}, where as the commercial formulation reaches that level at 7.8-mg L^{-1} (Figures 18, 19). Sabater and Carrasco (1997) determined that *Chlorella sp.* had an EC_{50} value (96 hr) of 54-mg L^{-1} when exposed to chlorsulfuron. EC_{50} values (7-day) for commercial formulations for Roundup, Aatrex, and Glean were calculated using the EPA Linear Interpolation Model software. (Table 24 and 28) Because the concentration ranges for Roundup (commercial) and atrazine (active ingredient) were not sufficiently high, values could not be determined. The calculated EC_{25} value suggests a dose-response of *P. oedogonia* to Roundup. These EC_{50} and EC_{25} values all fall within published ranges (Fletcher et al. 1996, Solomon, et al. 1996, Geisy, et al. 2000) although published values are higher than determined by this study (Table 23

When exposed to Glean, *P. oedogonia* does not show the hormetic effect seen in the field study. Field results showed increased growth of periphyton at low concentrations of Glean. Wendt-Rasch et al. (2003a) suggests that bottle tests can overestimate the toxicity of herbicides compared to field experiments. Because bottle tests use only one organism, that organism is either tolerant or intolerant to chemical exposure. If the organism is intolerant, growth rates will be reduced.

Published values of EC_{50} (Table 25) suggest periphyton and macrophytes are sensitive to sulfonylurea herbicides (Nystrom et al., 1999). Coyner et al. (2001) reported that 1ppb of Glean reduced new leaf production in *Potamogeton pectinatus*. Sabter et al. (2002) determined that freshwater algae have a chlorsulfuron EC_{50} (96 hour) value of 105 mg L^{-1}. The EC_{50} value (6.0 mg L^{-1} for 7 days) is considerable less as determined by this study. This would seem to indicate that the periphyton used in this study is more sensitive to Glean and chlorsulfuron than those species previously.

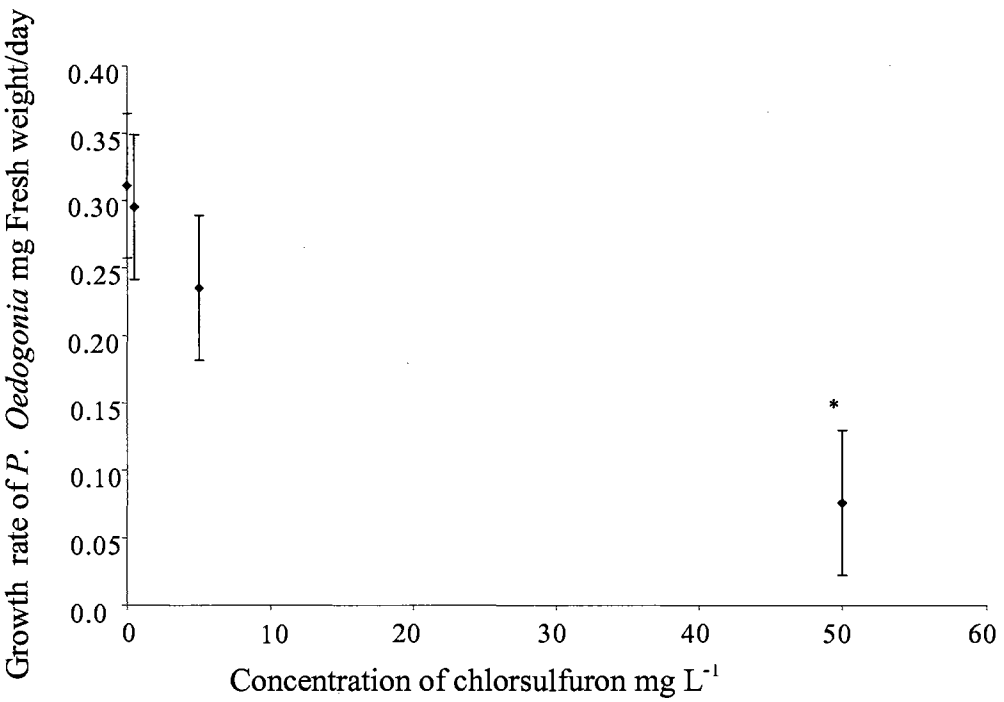

Figure 18. Effects of chlorsulfuron on the growth rate of *Pithophora oedogonia*. Error bars represent the standard error of the mean and the * indicates significantly different from the control at $p < 0.05$.

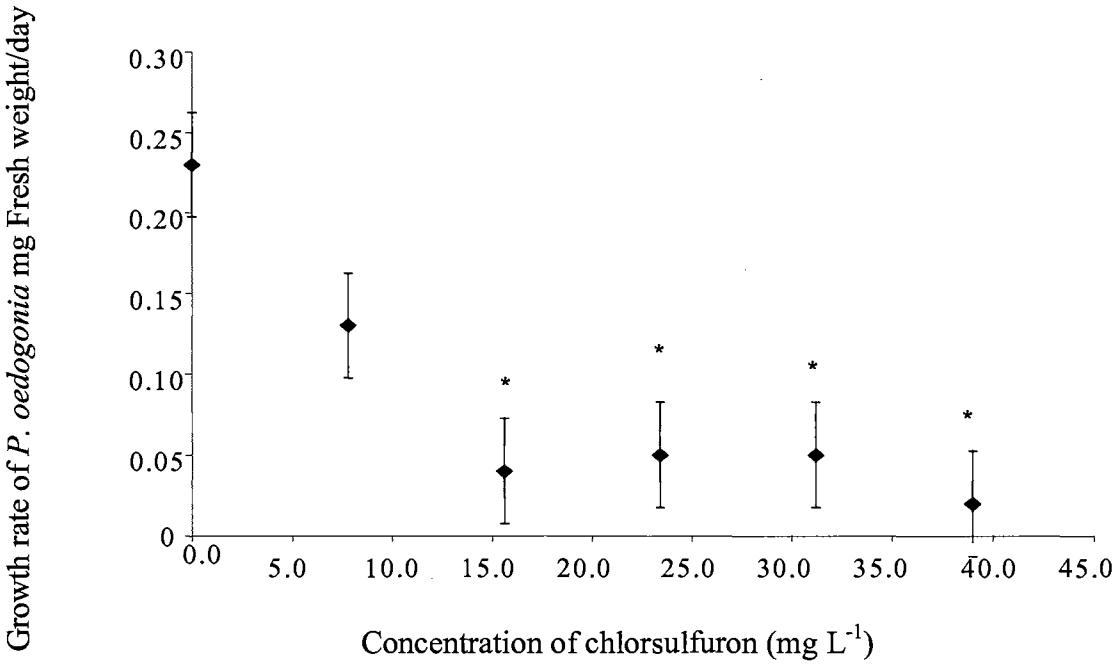

Figure 19. Effects of Glean on the growth rate of *Pithophora oedogonia.* An error bar represents the standard error of the mean and the * indicates significantly different from the control at $p<0.05$.

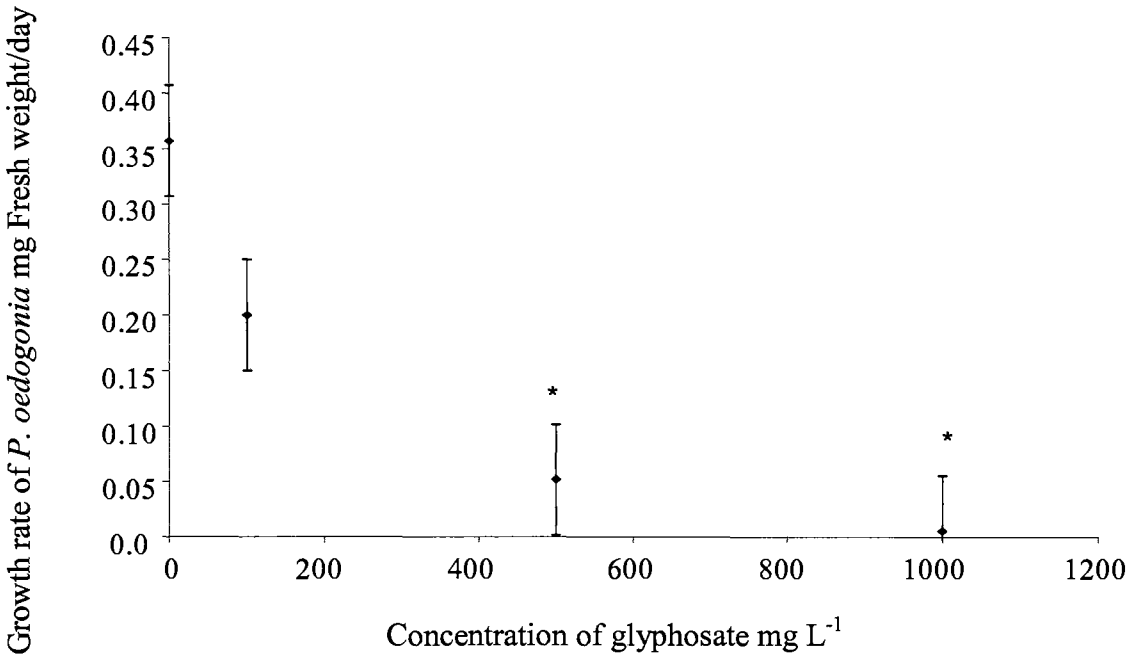

Figure 20. Effects of glyphosate on the growth rate of *Pithophora oedogonia*. An eerror bar represents the standard error of the mean and the * indicates significantly different from the control at $p< 0.05$.

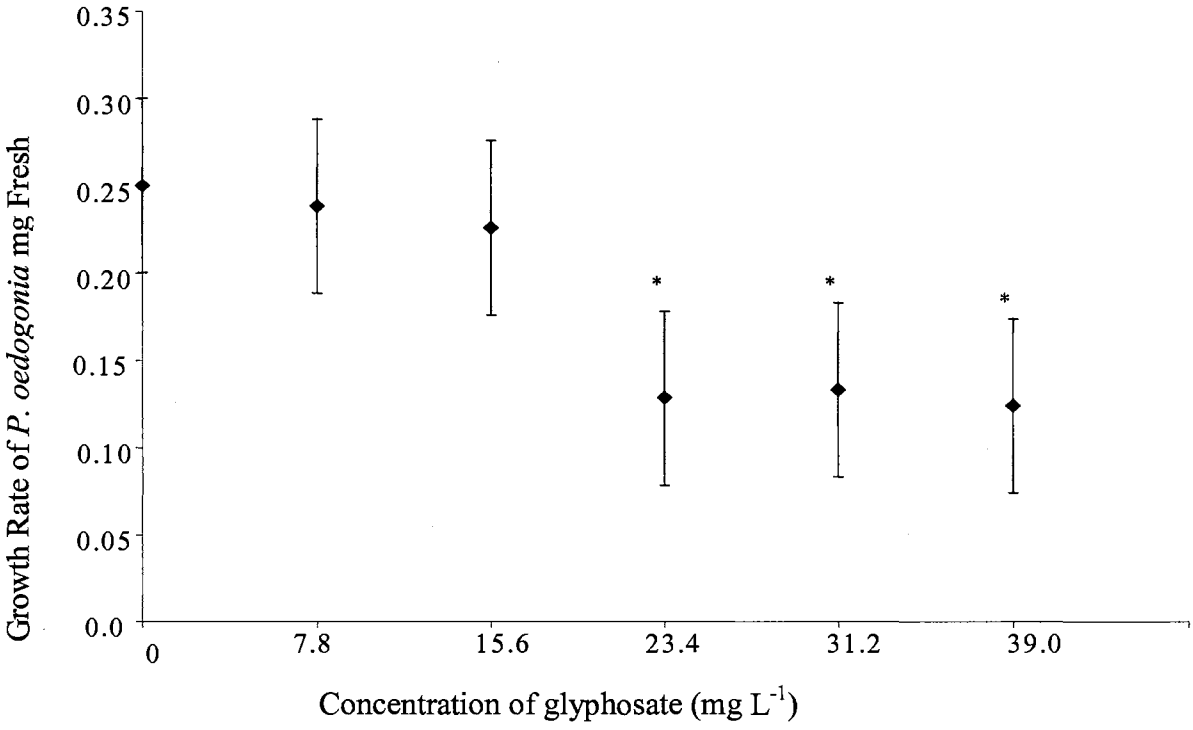

Figure 21. Effects of Roundup on the growth rate of *Pithophora oedogonia*. An error bar represents the standard error of the mean and the * indicates significantly different from the control at $p< 0.05$.

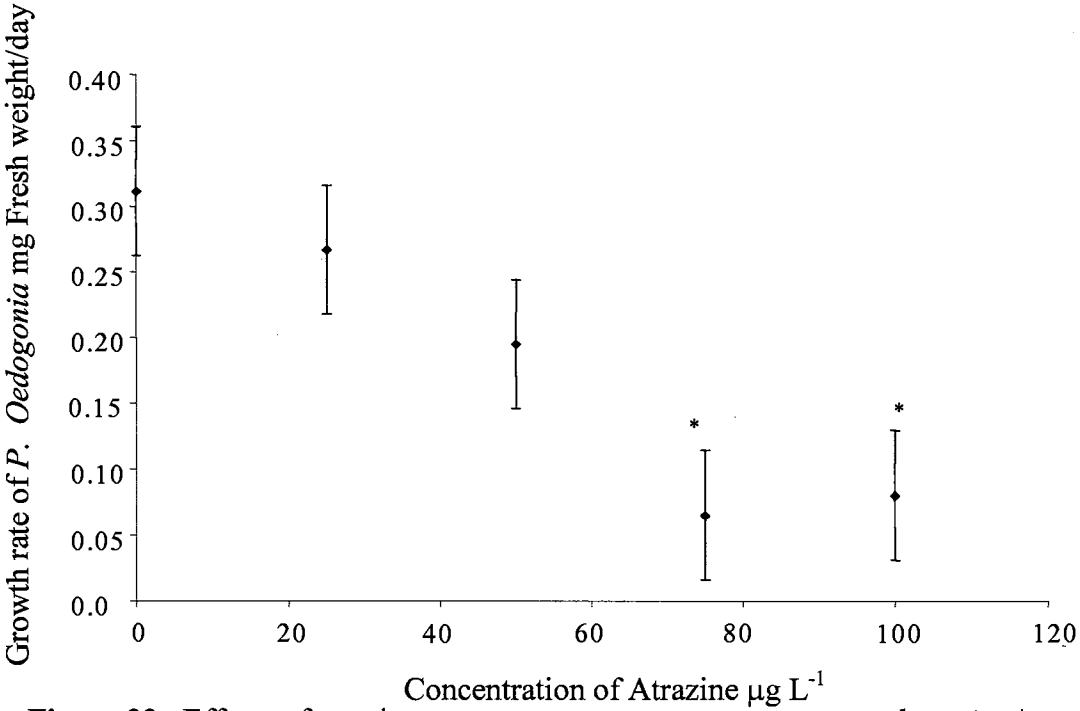

Figure 22. Effects of atrazine on the growth rate of *Pithophora oedogonia*. An error bar represents the standard error of the mean and the * indicates significantly different from the control at $p < 0.05$.

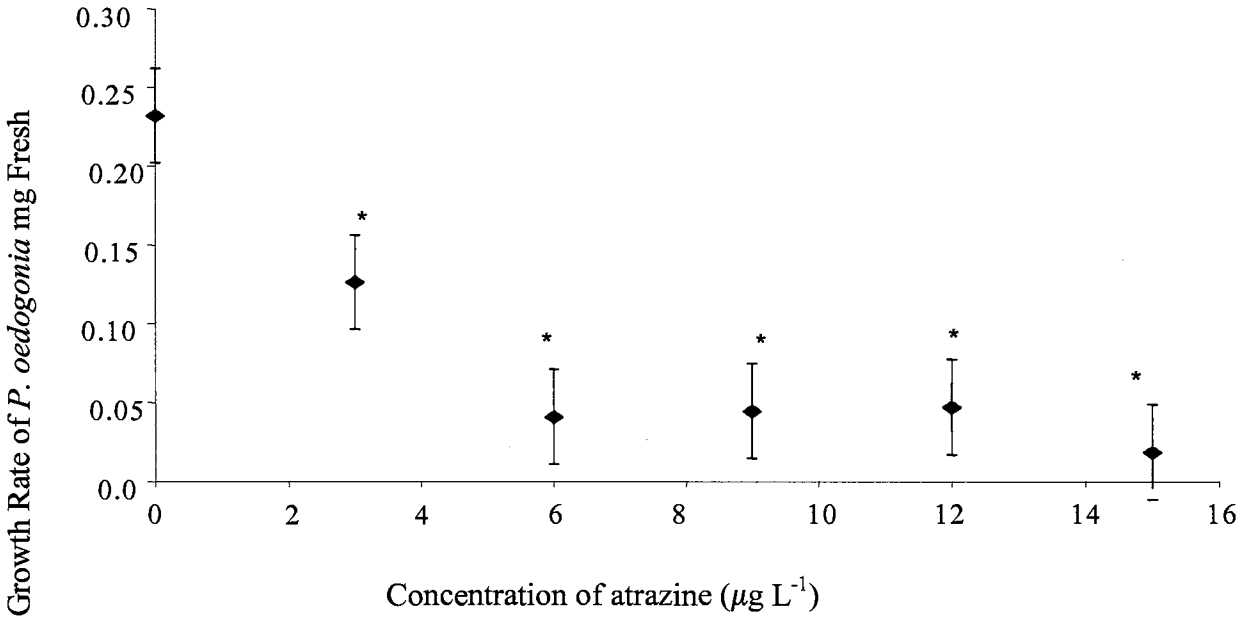

Figure 23. Effects of Aatrex on the growth rate of *Pithophora oedogonia*. An error bar represents the standard error of the mean and the * indicates significantly different from the control at $p<0.05$

The results suggest that commercial herbicides, which contain both the active ingredient and the surfactant, are effective in inhibiting growth of algae by two different means. The first is the active ingredient, which is designed to prevent growth and the second is the surfactant, which is designed to carry the active ingredient across the plant cell membrane. Geisy et al. (2000), however, determined that the surfactant in Roundup alone is toxic to plants.

Surfactant Study

As a separate investigation, effects on growth rates by the surfactant X-77 were determined. X-77 is one of many surfactants for glyphosate, being added to Rodeo® for use in aquatic environments (Tsui and Chu, 2003). X-77 is an alkylphenol ethoxylate that is used to conduct the herbicide into the plant by passing through the plant cell wall and the cell membrane. Figure 24 indicates surfactant produced a dose-response effect on growth rate that was similar to the commercial and active ingredient studies (Figures 18-23). Table 27 showed that at any of the concentrations of surfactant tested there was a reduction of growth rate. This agrees with Simenstad et al. (1996) and Hense et al. (2003) who reported significant reductions of algae from X-77 with exposure from 72 hours and 14 days respectively. Hense et al. (2003) further reported an EC_{50} of 0.98 mg L^{-1} from bottle tests using *Scenedesmus subspicatus.*

Figure 24 also shows the effects on growth rate by the surfactant X-77. The surfactant in this case does have a significant effect on growth rates. Mann and Bidwell (2001) have also found that the surfactant used in Roundup had a

significant effect on frog survival in the laboratory. Giesy et al. (2000) also reports the surfactant from Roundup can affect growth of invertebrates.

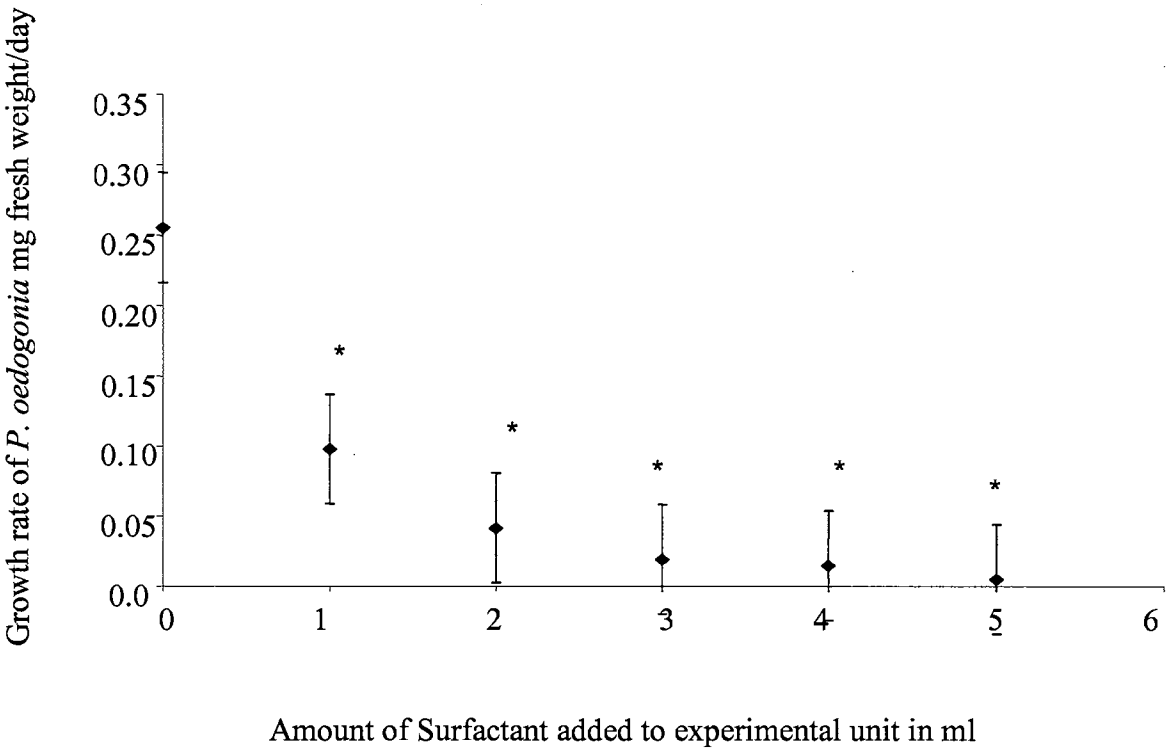

Figure 24. Effects of surfactant X-77 on growth rate of *Pithophora oedogonia*. An error bar represents the standard error of the mean and the * indicates significantly different from the control at $p<0.05$

Bottle Test for the effects of mixing active ingredients upon *Pithophora oedogonia*

Agricultural pesticide practices include mixing pesticides (Miller and Donahue, 1995). Pesticides are found in waterways and mixtures of these herbicides are becoming more frequent (USEPA, 1994). Mixtures of glyphosate and chlorsulfuron are being used in Western Oklahoma to control weed species in winter wheat fields (Roger Penner, personal communication). The herbicide

Fieldmaster® sold by the Monsanto Co. (St. Louis, MO) is a mixture of glyphosate and atrazine (Monsanto Co. St. Louis, MO). There are few studies in the literature that investigate the impact of mixtures upon algae (Hartgers et al., 1998, Christensen et al., 2001, Wendt-Rausch et al., 2003a,b). This study used a mixture of each herbicide at its EC_{50} because this provides a measurable response for the single herbicide and the mixtures of those herbicides.

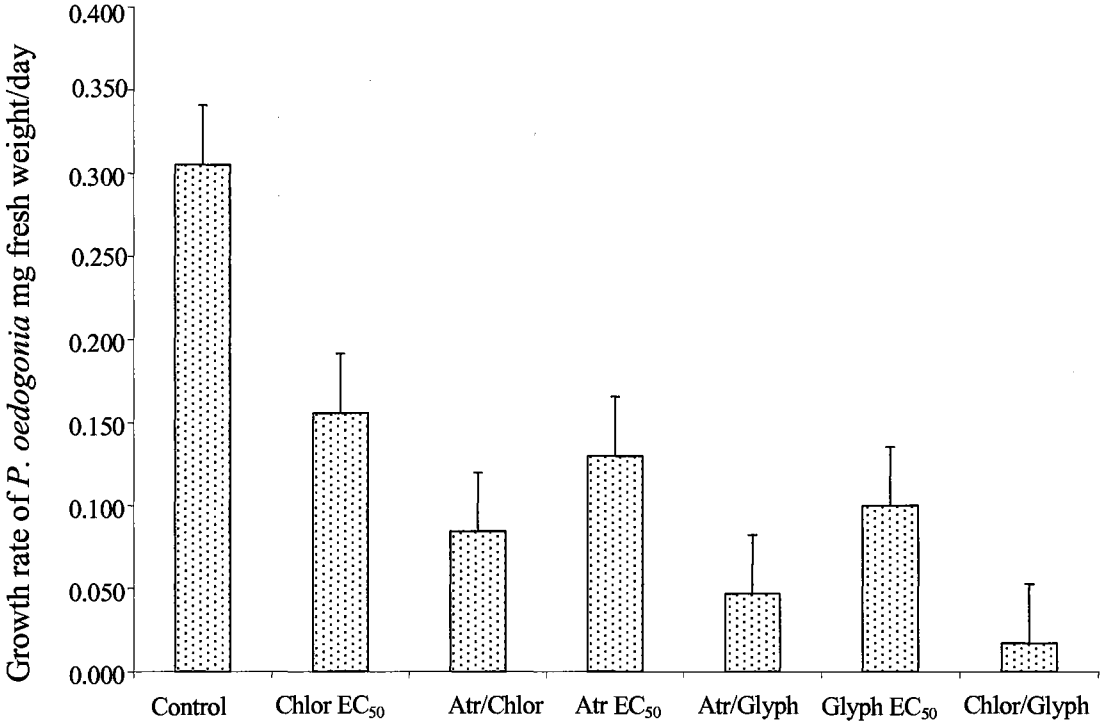

Figure 25. The Effect of herbicide mixture on growth rate of *Pithophora oedogonia* when exposed to tandem active ingredient at the EC_{50} level for each active ingredient. Growth rates at the EC_{50} value for each active ingredient is included to give a comparison to the mixture growth rate. Error bars are standard error of the mean.

Tables 25 and Figure 25 show the results of exposure to the mixtures, suggesting a stronger reduction in growth rates. Figure 25 shows the growth rate of *P. oedogonia* at the EC_{50} value for each individual active ingredient and pairwise

mixtures. The mixtures have a more pronounced effect than the individual active ingredients. Hartgers et al. (1998) determined that mixtures of herbicides reduced photosynthetic efficiency upon initial exposure. This study also determined that there was a community shift in primary producers upon exposure to herbicide mixtures.

The effects of herbicides in mixtures are described as synergistic, additive, or antagonistic (Timbrell, 1995). Synergistic is defined as the sum of the toxic components greater than the individual components, additive as a response equal to the addition of individual components, and antagonistic as the effects of the two toxic components working against each other (Timbrell, 1995). Marking (1977) described the above terms as non-quantitative and developed a quantitative measurement for mixtures, the Cumulative-Sum-Additive Index (CSAI). The CSAI is also better able to quantify the value as greater than additive, or less than additive contributions for two chemicals (Marking 1977). The CSAI measures the additive nature of the chemicals by assigning 0.00 an additive value, >0.00 greater than additive, and <0.00 less than additive. The index assumes that two chemicals when placed in tandem are additive (Marking, 1977. The results for the CSAI are found in Table 24 and Table 34.

Table 34 indicates the effects of the mixtures on growth rates of *P. oedogonia* if the effect were additive. Using the expected growth rate if herbicide exposure were additive and the actual growth rates, the response to the mixtures can be seen.

Table 34. Comparison of expected growth rates to actual growth rates per day for *P. oedogonia* exposed to herbicide mixtures. Expected growth rates and percentages of EC_{50} values calculated from Figure 21 and Table 16.

	Percent of Actual Growth Rates at EC_{50} Values if Additive	Expected Growth Rate of *P. oedogonia* if herbicide action were Additive	Actual Growth Rate	CSAI
		--mg fresh weight/day--		
Chlor/Atr	20	0.06	0.09	-0.19
Chlor/Glyph	17	0.05	0.02	0.64
Atr/Glyph	14	0.04	0.04	0.28

The results suggest that there are reductions in the overall growth of the test algae when exposed to mixtures of herbicides. The greatest reduction in growth was seen with the chlorsulfuron/ glyphosate mixture. The CSAI value was calculated at 0.64, which is a greater-than-additive or synergistic effect. Chlorsulfuron and glyphosate both affect the production of amino acids that can be used in cell division and cell wall synthesis of plants (Goodwin and Mercer, 1990). Interruption of these cell processes would have a detrimental effect on periphyton. The mixture of atrazine and glyphosate had a CSAI value of 0.28 suggesting greater-than-additive (additive) effect but not as strong as the chlorsulfuron/glyphosate tandem. Because each herbicide affect different biochemical pathways there are two different pathways being inhibited. Finally the tandem of atrazine/chlorsulfuron produced a CSAI value of –0.19, which indicates that there is a less than additive (antagonistic) effect upon the growth of the algae. An antagonistic effect would mean the herbicides are working against or moderate the effect of the other. Christensen et al. (2001) indicates that atrazine is an antidote to the herbicide decylamine when they are mixed.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

Agriculturally (environmentally) relevant concentrations of Aatrex, Glean, and Roundup have no significant effect upon total chlorophyll content of a mixed community of periphyton in the stream tested. The results of this study, measuring chlorophyll *a*, chlorophyll *b*, and chlorophyll *c*, and determining the ratio between this pigments, suggests the herbicide Aatrex shifts periphyton communities toward Chlorophyta and Cyanophyta vs Chrysophyta, and the herbicides Glean and Roundup shift periphyton communities toward Chlorophyta and Chrysophyta vs Cyanophyta. This information could be used to predict the changes in periphyton communities. Chlorophyll species ratios should give an indication in the shift of periphyton species in streams that these herbicides have been introduced. This initial test can save time in determining the impact of a transient exposure to herbicides. The identification of chyrsophytes in particular, can be a time consuming process and requires individual trained in diatom identification.

When exposed to the herbicide Glean at low concentrations, chlorophytes and chrysophytes increase based upon ratio of chlorophyll *b* and *c* to chlorophyll *a* concentrations. The increase in concentration could be due to hormesis, a stimulatory process at low chemical concentrations, or it could be that at low concentration chlorophytes and chrysophytes are favored, and competitors are

eliminated. As concentrations of Glean increase, the favored organisms would be equally reduced and the chlorophyll ratios would become more like the control. Because there are no significant changes in total chlorophyll at higher exposures, the effects of Aatrex, Glean, and Roundup and their active ingredients may be seen at the grazer and higher levels.

This study determined that the Matlock Periphytometer could be used to assess the impact of herbicides on periphyton *in situ*. Matlock et al. (1998) hypothesized that it would be possible to add herbicides to the periphytometer bottle and measure impact upon a periphyton community. I added a rubber septum for injection of herbicides and changed the bottle to an amber color to prevent photodegradation. Procedural changes included allowing the periphytometer to recruit periphyton for the first week and then adding herbicides the second week to assess changes in the community.

Bottle tests showed active ingredients, commercial formulations, and surfactants all cause reductions in growth rates of *P. oedogonia*. These tests showed that *P. oedogonia* responds to herbicides in a similar manner as other algal test organisms. This response includes lower growth rates at increasing concentrations of herbicide, but no effect of herbicides on the concentration of chlorophyll in cells (μg chlorophyll/mg of fresh weight). The observation that chlorophyll concentration of cells does not decrease significantly with exposure, allows mg of chlorophyll per unit time to be used as a surrogate measure for growth rate in these studies.

By comparing the results of commercial formulations and the active ingredient of those commercial formulations, it appears that commercial formulations are more effective at reducing growth rates of *P. oedogonia*. Commercial herbicide formulations have surfactants added and my study indicated that active ingredients and surfactant each had a dose-response effect. The commercial formulation of the herbicides, however, had a greater dose-response effect upon the algae based upon growth curves.

Active ingredients affect growth rates differently when mixed together than individually. The results shown here indicate that there are reductions in growth rates at considerably higher rates than the individual active ingredients alone. Again, as with the single herbicide bottle test, the growth rate was the only variable affected. The amount of chlorophyll per mg of fresh weight of *P. oedogonia* was statistically unchanged from the control. The Cumulative Sum Additive Index, which is a quantitative measure of the effects of mixtures upon growth rate, can describe the toxicity of mixtures. Components of a mixture can have less than additive or antagonistic effect (working against one another), an additive effect, (working with each other), or a greater than additive or synergistic effect (greatly adding to each other). The atrazine + chlorsulfuron mixture had a less than additive or antagonistic effect, atrazine + glyphosate showed an additive effect, and glyphosate + chlorsulfuron showed a greater than additive or synergistic effect upon growth rate of *P. oedogonia*.

Recommendations

Recommendations for further study include:

1) Determine the change in communities by measuring the chlorophyll species during different growing seasons with exposure to herbicides and check for differences in chlorophyll ratios between seasons.
2) Determine if the growth of periphyton exposed to low concentrations of Glean produce is a hormetic effect, and characterize the effect as to whether eukaryotic periphyton are stimulated into growth or if prokaryotic periphyton are simply inhibited at low concentrations giving a higher chlorophyll *b/a* ratio.
3) Repeat the field experiment and bottle tests using mixtures of commercial herbicides to determine if
    a) There are changes in community structure as measured by changes in chlorophyll species and if
    b) A dose-response from commercial herbicide mixtures similar to individual commercial herbicide results.
4) Accurate analysis of chemical concentrations used in the field and the bottle tests.

Additional recommendations include studying photosynthesis of *P. oedogonia* for immediate effects of herbicides. Although the cellular content of chlorophyll was not reduced significantly, photosynthesis could have been affected by exposure to herbicides.

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APPENDIX A
GLASS FILTER PHOTOGRAPHS

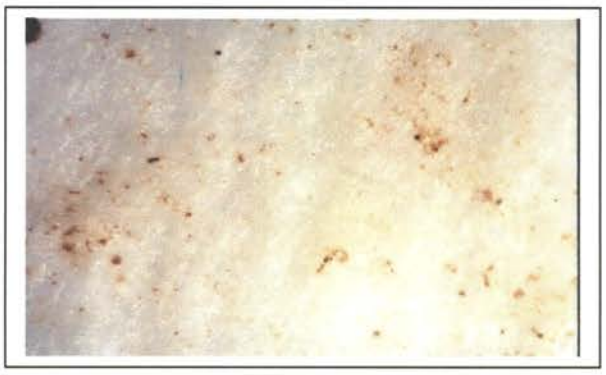

Figure 26. $CuSO_4$ glass filter total magnification 150X.

Figure 27. Aatrex control glass filter total magnification 150X

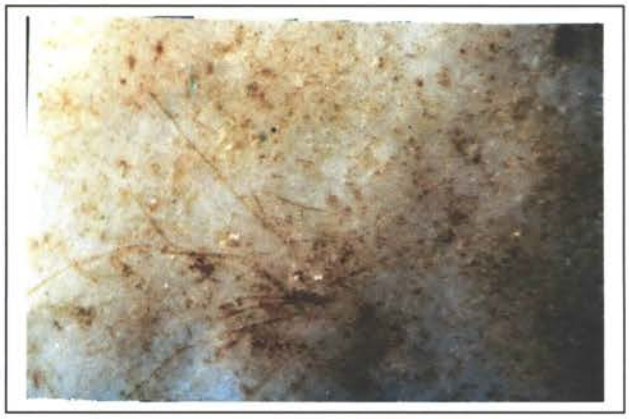

Figure 28. Aatrex 1ml glass filter total magnification 150X.

Figure 29. Aatrex 2ml glass filter total magnification 150X.

Figure 30. Aatrex 4ml glass filter total magnification 150X

Figure 31. Aatrex 5ml glass filter total magnification 150X

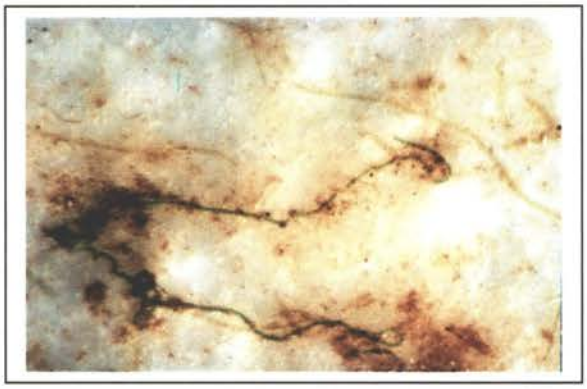

Figure 32. Glean Control glass filter total magnification 150X

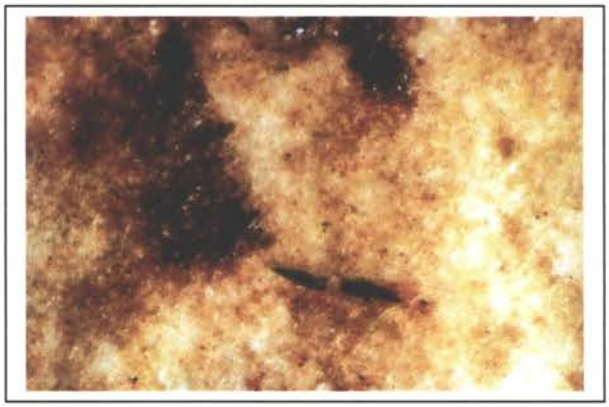

Figure 33. Glean 1ml glass filter total magnification 150X.

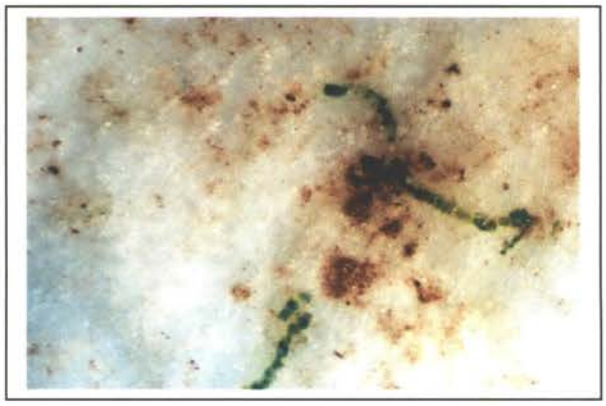

Figure 34. Glean 2ml glass filter total magnification 150X

Figure 35. Glean 3 ml glass filter total magnification 150X

Figure 36. Glean 4ml glass filter total magnification 150X

Figure 37. Glean 5ml Glass filter total magnification 150X

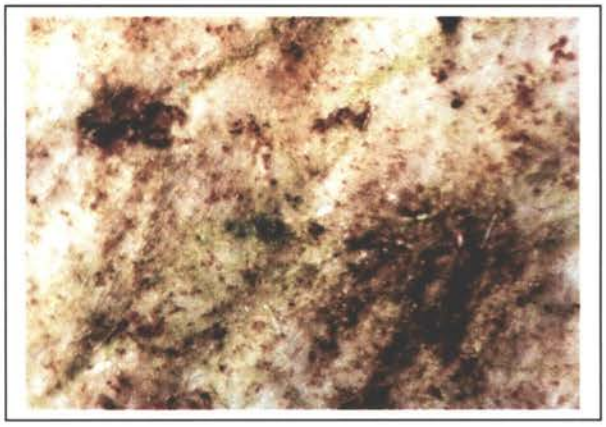

Figure 38. Roundup control glass filter total magnification 150X

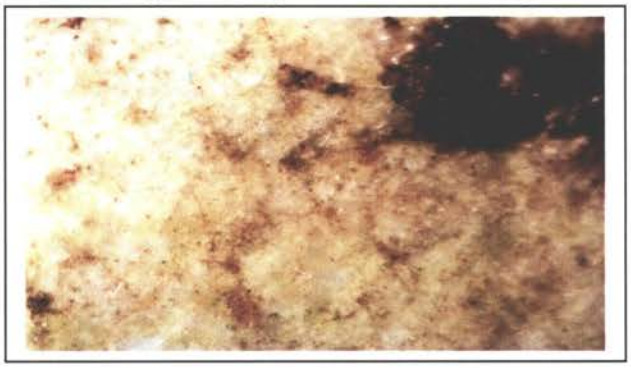

Figure 39. Roundup 1 ml Glass Filter total magnification 150X

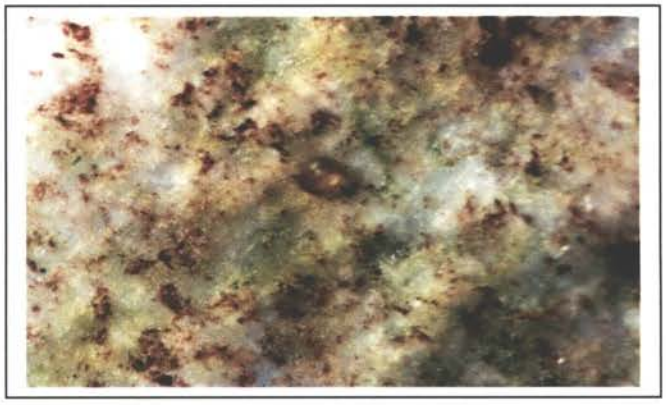

Figure 40. Roundup 2ml Glass filter total magnification 150X

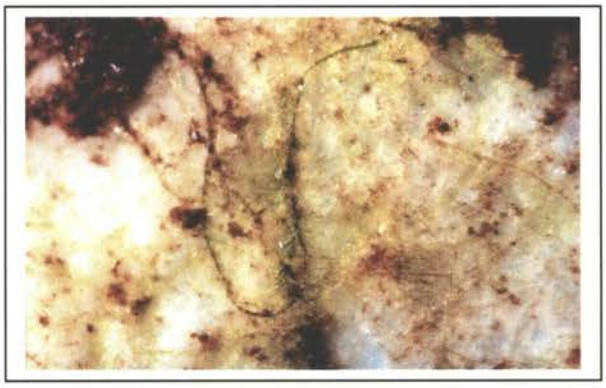

Figure 41. Roundup 3ml Glass Filter total magnification 150X

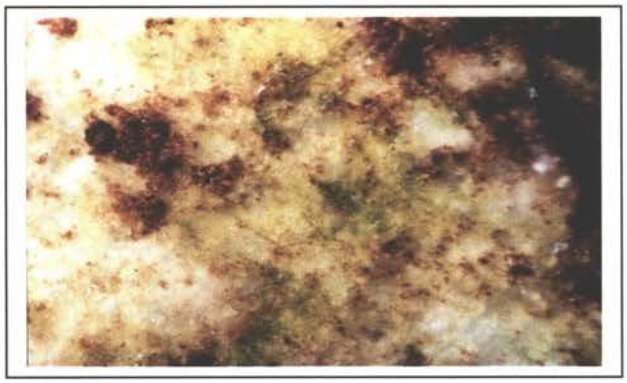

Figure 42. Roundup 4ml Glass Filter total magnification 150X

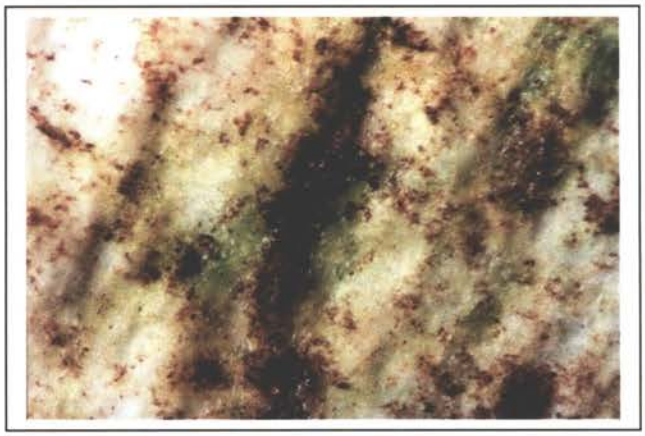

Figure 43. Roundup 5 ml Glass Filter total magnification 150X

APPENDIX B

STATISICAL ANALYZES OF DATA

Section 1: Effects of formulated Roundup, Aatrex and Glean on *Pithophora oedogonia*

ANOVA Table of Dependent Variable: growth rate and the effect of all herbicides

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	41	2.16	0.05	5.36	<.0001
Error	127	1.25	0.001		
Corrected Total	168	3.42			

ANOVA Split-plot analysis of the formulated herbicides upon growth rate as the dependent variable

Source	DF	Type I SS	Mean Square	F Value	Pr > F□
HERB	2	0.45	0.22	23.13	<.0001
DATE(HERB)	4	0.22	0.06	5.71	0.0003
CONC	5	1.05	0.21	21.33	<.0001
HERB*CONC	10	0.35	0.04	3.55	0.0004
DATE*CONC(HERB)	20	0.08	0.004	0.42	0.9869

Source	DF	Type III SS	Mean Square	F Value	Pr > F
HERB	2	0.53	0.26	26.83	<.0001
DATE(HERB)	4	0.26	0.07	6.69	<.0001
CONC	5	0.97	0.19	19.69	<.0001
HERB*CONC	10	0.35	0.04	3.55	0.0004
DATE*CONC(HERB)	20	0.08	0.004	0.42	0.9869

Tests of Effect Slices multiple comparison test using growth rate of *P. oedogonia* as the dependent variable. This test is determining which herbicide at all concentrations and all concentrations of all herbicides have impacts upon the algae

Effect	HERB	CONC	DF	DF	F Value	Pr > F
HERB*CONC	Aatrex		5	147	9.71	<.0001
HERB*CONC	Glean		5	147	13.97	<.0001
HERB*CONC	Roundup		5	147	7.06	<.0001
HERB*CONC		0	2	7.69	0.43	0.67
HERB*CONC		1	2	10.5	10.28	0.003
HERB*CONC		2	2	10.5	6.42	0.015
HERB*CONC		3	2	10.5	1.49	0.27
HERB*CONC		4	2	10.5	0.35	0.71
HERB*CONC		5	2	10.5	2.78	0.11

Type 3 Tests of Fixed Effects for the formulated bottle tests using growth rate

Effect	DF	DF	F Value	Pr > F
HERB	2	3.91	3.61	0.1293
CONC	5	147	21.58	<.0001
HERB*CONC	10	147	3.86	0.0001

Split-plot ANOVA Analysis of formulated bottle tests using as a Dependent Variable: ug chl a/ mg Fresh weight *P. oedogonia*

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	41	39.2	0.96	5.41	<.0001
Error	127	22.5	0.18		
Corrected Total	168	61.7			

Least Squares Means multiple comparison test using ug chl a/ mg Fresh weight *P. oedogonia* as the dependent variable. This test is determining which herbicide at all concentrations and all concentrations of all herbicides have impacts upon the algae.

Effect	HERB	CONC	Estimate	Error	DF	t Value	Pr > |t|
HERB	Aatrex		1.40	0.30	4.01	4.65	0.01
HERB	Glean		1.03	0.37	3.96	2.79	0.05
HERB	Roundup		1.04	0.37	3.97	2.84	0.05
CONC		0	1.02	0.21	5.18	4.74	0.005
CONC		1	1.08	0.22	5.65	4.91	0.003
CONC		2	1.23	0.22	5.65	5.59	0.002
CONC		3	1.09	0.22	5.65	4.96	0.003
CONC		4	1.2	0.22	5.65	5.67	0.002
CONC		5	1.29	0.22	5.65	5.88	0.001

Tests of Effect Slices multiple comparison test using ug chl a/ mg Fresh weight *P. oedogonia* as the dependent variable. This test is determining which herbicide at all concentrations and all concentrations of all herbicides have impacts upon the algae

Effect	HERB	CONC	DF	DF	F Value	Pr > F
HERB*CONC	Aatrex		5	22.6	2.03	0.11
HERB*CONC	Glean		5	16.8	1.21	0.34
HERB*CON	Roundup		5	18.6	0.10	0.99
HERB*CONC		0	2	5.19	0.03	0.97
HERB*CONC		1	2	5.66	0.82	0.48
HERB*CONC		2	2	5.66	0.30	0.75
HERB*CONC		3	2	5.66	0.84	0.48
HERB*CONC		4	2	5.66	0.18	0.84
HERB*CONC		5	2	5.66	0.71	0.53

Type 3 Tests of Fixed Effects using ug chl a/ mg Fresh weight

Effect	DF	DF	F Value	Pr > F
HERB	2	3.98	0.42	0.6838
CONC	5	19	1.38	0.2742
HERB*CONC	10	19.1	0.88	0.5702

Tukey multiple (HSD) comparison of all herbicides and all concentrations of herbicides using growth rate as the dependent variable. All herbicide concentrations are compared to each other to determine significance.

HERB	CONC	HERB	CONC	Est	Error	DF	t Value	Pr > |t|	Adjustment	Adj P
Aatrex	0	Aatrex	1	0.21	0.039	147	5.49	<.0001	Tukey-Kramer	
<.0001	Aatrex	0	Aatrex	2	0.21	0.04	147	5.40	<.0001	Tukey-
Kramer	<.0001	Aatrex	0	Aatrex	3	0.17	0.04	147	4.30	<.0001
Tukey-Kramer	0.0040									
Aatrex	0	Aatrex	4	0.18	0.04	147	4.74	<.0001	Tukey-Kramer	0.0007
Aatrex	0	Aatrex	5	0.18	0.04	147	4.77	<.0001	Tukey-Kramer	0.0006
Aatrex	1	Aatrex	2	-0.004	0.04	147	-0.09	0.93	Tukey-Kramer	1.0000
Aatrex	1	Aatrex	3	-0.05	0.04	147	-1.09	0.28	Tukey-Kramer	0.9998
Aatrex	1	Aatrex	4	-0.03	0.04	147	-0.69	0.49	Tukey-Kramer	1.0000
Aatrex	1	Aatrex	5	-0.03	0.04	147	-0.66	0.51	Tukey-Kramer	1.0000
Aatrex	2	Aatrex	3	-0.043	0.04	147	-1.00	0.31	Tukey-Kramer	0.9999
Aatrex	2	Aatrex	4	-0.023	0.04	147	-0.60	0.5507	Tukey-Kramer	1.0000
Aatrex	2	Aatrex	5	-0.02	0.04	147	-0.57	0.5696	Tukey-Kramer	1.0000
Aatrex	3	Aatrex	4	0.02	0.04	147	0.41	0.6855	Tukey-Kramer	1.0000
Aatrex	3	Aatrex	5	0.02	0.04	147	0.43	0.6650	Tukey-Kramer	1.0000
Aatrex	4	Aatrex	5	0.001	0.04	147	0.03	0.9776	Tukey-Kramer	1.0000
Glean	0	Glean	1	0.17	0.04	148	3.99	0.0001	Tukey-Kramer	0.0121
Glean	0	Glean	2	0.2	0.04	148	5.60	<.0001	Tukey-Kramer	<.0001
Glean	0	Glean	3	0.29	0.04	148	6.71	<.0001	Tukey-Kramer	<.0001
Glean	0	Glean	4	0.12	0.04	148	4.67	<.0001	Tukey-Kramer	0.0009
Glean	0	Glean	5	0.28	0.04	148	6.58	<.0001	Tukey-Kramer	<.0001
Glean	1	Glean	2	0.069	0.04	147	1.44	0.15	Tukey-Kramer	0.9931
Glean	1	Glean	3	0.12	0.04	147	2.43	0.01	Tukey-Kramer	0.5861
Glean	1	Glean	4	0.03	0.04	147	0.61	0.54	Tukey-Kramer	1.0000
Glean	1	Glean	5	0.11	0.04	147	2.32	0.02	Tukey-Kramer	0.6690
Glean	2	Glean	3	0.05	0.04	147	0.99	0.32	Tukey-Kramer	0.9999
Glean	2	Glean	4	-0.04	0.04	147	-0.83	0.41	Tukey-Kramer	1.0000
Glean	2	Glean	5	0.04	0.04	147	0.88	0.38	Tukey-Kramer	1.0000
Glean	3	Glean	4	-0.09	0.04	147	-1.82	0.07	Tukey-Kramer	0.9343
Glean	4	Glean	5	0.08	0.04	147	1.71	0.09	Tukey-Kramer	0.9623
Roundup	0	Roundup	1	-0.039	0.04	147	-0.85	0.40	Tukey-Kramer	1.0000
Roundup	0	Roundup	2	0.032	0.04	147	0.71	0.48	Tukey-Kramer	1.0000
Roundup	0	Roundup	3	0.16	0.04	147	3.50	0.001	Tukey-Kramer	0.06
Roundup	0	Roundup	4	0.17	0.04	147	3.87	0.0002	Tukey-Kramer	0.02
Roundup	0	Roundup	5	0.11	0.04	147	2.52	0.013	Tukey-Kramer	0.52
Roundup	1	Roundup	2	0.07	0.04	147	1.48	0.14	Tukey-Kramer	0.99
Roundup	1	Roundup	3	0.20	0.04	147	4.13	<.0001	Tukey-Kramer	0.007
Roundup	1	Roundup	4	0.21	0.04	147	4.48	<.0001	Tukey-Kramer	0.002
Roundup	1	Roundup	5	0.15	0.04	147	3.20	0.0017	Tukey-Kramer	0.13
Roundup	2	Roundup	3	0.13	0.04	147	2.65	0.0090	Tukey-Kramer	0.43
Roundup	2	Roundup	4	0.14	0.04	147	3.00	0.003	Tukey-Kramer	0.21
Roundup	2	Roundup	5	0.08	0.04	147	1.72	0.09	Tukey-Kramer	0.96
Roundup	3	Roundup	4	0.017	0.04	147	0.35	0.73	Tukey-Kramer	1.000
Roundup	3	Roundup	5	-0.04	0.04	147	-0.93	0.35	Tukey-Kramer	1.000
Roundup	4	Roundup	5	-0.06	0.04	147	-1.28	0.20	Tukey-Kramer	0.9982

Tukey multiple (HSD) comparison of all herbicides and all concentrations of herbicides using ug chlorophyll *a* per mg fresh weight as the dependent variable. All herbicide concentrations are compared to each other to determine significance.

HERB CONC	HERB CONC	Est	Error	DF	t Value	Pr > |t|	Adjustment	Adj P
Aatrex 0	Aatrex 1	-0.41	0.20	19.5	-2.02	0.06	Tukey-Kramer	0.827
Aatrex 0	Aatrex 2	-0.41	0.20	19.5	-2.03	0.06	Tukey-Kramer	0.821
Aatrex 0	Aatrex 3	-0.42	0.20	19.5	-2.07	0.05	Tukey-Kramer	0.805
Aatrex 0	Aatrex 4	-0.35	0.20	19.5	-1.70	0.10	Tukey-Kramer	0.943
Aatrex 0	Aatrex 5	-0.60	0.20	19.5	-2.96	0.008	Tukey-Kramer	0.303
Aatrex 1	Aatrex 2	-0.002	0.22	25.4	-0.01	0.99	Tukey-Kramer	1.000
Aatrex 1	Aatrex 3	-0.009	0.22	25.4	-0.04	0.97	Tukey-Kramer	1.000
Aatrex 1	Aatrex 4	0.065	0.22	25.4	0.30	0.77	Tukey-Kramer	1.000
Aatrex 1	Aatrex 5	-0.192	0.22	25.4	-0.88	0.39	Tukey-Kramer	0.999
Aatrex 2	Aatrex 3	-0.007	0.22	25.4	-0.03	0.98	Tukey-Kramer	1.000
Aatrex 2	Aatrex 4	0.067	0.22	25.4	0.31	0.76	Tukey-Kramer	1.000
Aatrex 2	Aatrex 5	-0.19	0.22	25.4	-0.87	0.39	Tukey-Kramer	1.000
Aatrex 3	Aatrex 4	0.074	0.22	25.4	0.34	0.74	Tukey-Kramer	1.000
Aatrex 3	Aatrex 5	-0.18	0.22	25.4	-0.84	0.41	Tukey-Kramer	1.000
Aatrex 4	Aatrex 5	-0.26	0.22	25.4	-1.18	0.25	Tukey-Kramer	0.998
Glean 0	Glean 1	0.11	0.22	14.1	0.49	0.63	Tukey-Kramer	1.00
Glean 0	Glean 2	-0.23	0.22	14.1	-0.97	0.35	Tukey-Kramer	0.999
Glean 0	Glean 3	0.12	0.22	14.1	0.54	0.60	Tukey-Kramer	1.000
Glean 0	Glean 4	-0.34	0.22	14.1	-1.46	0.17	Tukey-Kramer	0.984
Glean 0	Glean 5	-0.19	0.22	14.1	-0.80	0.44	Tukey-Kramer	1.000
Glean 1	Glean 2	-0.34	0.22	19.8	-1.37	0.19	Tukey-Kramer	0.991
Glean 1	Glean 3	0.01	0.25	19.8	0.04	0.97	Tukey-Kramer	1.000
Glean 1	Glean 4	-0.46	0.25	19.8	-1.82	0.08	Tukey-Kramer	0.908
Glean 1	Glean 5	-0.30	0.25	19.8	-1.21	0.24	Tukey-Kramer	0.998
Glean 2	Glean 3	0.35	0.25	19.8	1.41	0.18	Tukey-Kramer	0.989
Glean 2	Glean 4	-0.11	0.25	19.8	-0.46	0.65	Tukey-Kramer	1.000
Glean 2	Glean 5	0.04	0.25	19.8	0.16	0.87	Tukey-Kramer	1.000
Glean 3	Glean 4	-0.46	0.25	19.8	-1.86	0.08	Tukey-Kramer	0.894
Glean 3	Glean 5	-0.31	0.25	19.8	-1.24	0.23	Tukey-Kramer	0.997
Glean 4	Glean 5	0.15	0.25	19.8	0.62	0.54	Tukey-Kramer	1.000
Roundup 0	Roundup 1	0.12	0.24	17	0.48	0.64	Tukey-Kramer	1.000
Roundup 0	Roundup 2	0.01	0.24	17	0.05	0.96	Tukey-Kramer	1.000
Roundup 0	Roundup 3	0.09	0.24	17	0.36	0.73	Tukey-Kramer	1.000
Roundup 0	Roundup 4	0.008	0.24	17	0.03	0.98	Tukey-Kramer	1.000
Roundup 0	Roundup 5	-0.03	0.24	17	-0.13	0.90	Tukey-Kramer	1.000
Roundup 1	Roundup 2	-0.10	0.25	19.8	-0.42	0.68	Tukey-Kramer	1.000
Roundup 1	Roundup 3	-0.03	0.25	19.8	-0.12	0.90	Tukey-Kramer	1.000
Roundup 1	Roundup 4	-0.11	0.25	19.8	-0.44	0.67	Tukey-Kramer	1.000
Roundup 1	Roundup 5	-0.15	0.25	19.8	-0.59	0.56	Tukey-Kramer	1.000
Roundup 2	Roundup 3	0.07	0.25	19.8	0.30	0.77	Tukey-Kramer	1.000
Roundup 2	Roundup 4	-0.004	0.25	19.8	-0.02	0.99	Tukey-Kramer	1.000
Roundup 2	Roundup 5	-0.042	0.25	19.8	-0.17	0.87	Tukey-Kramer	1.000
Roundup 3	Roundup 4	-0.08	0.25	19.8	-0.31	0.76	Tukey-Kramer	1.000
Roundup 3	Roundup 5	-0.12	0.25	19.8	-0.47	0.65	Tukey-Kramer	1.000
Roundup 4	Roundup 5	-0.04	0.25	19.8	-0.15	0.88	Tukey-Kramer	1.000

Split-Plot Analysis of Matlock Periphytometer experiment using Chlorophyll *a,b,c*, and total Chlorophyll as dependent variables

Source	DF	Sum Squares	Mean Square	F Value	Pr > F
Model	35	8.99	0.257	14.39	<.0001
Error	141	2.52	0.018		
Corrected Total	176	11.50			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
HERB	2	1.71	0.86	47.92	<.0001
DATE(HERB)	3	5.12	1.70	95.65	<.0001
CONC	5	0.56	0.11	6.28	<.0001
HERB*CONC	10	0.65	0.07	3.66	0.0002
DATE*CONC(HERB)	15	0.943	0.06	3.52	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
HERB	2	1.18	0.59	33.15	<.0001
DATE(HERB)	3	5.17	1.72	96.59	<.0001
CONC	5	0.42	0.09	4.74	0.0005
HERB*CONC	10	0.43	0.04	2.44	0.0104
DATE*CONC(HERB)	15	0.94	0.06	3.52	<.0001

Split-Plot Analysis of Matlock Periphytometer experiment using Chlorophyll *a* as dependent variables

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	35	3.39	0.097	10.43	<.0001
Error	141	1.31	0.009		
Corrected Total	176	4.70			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
HERB	2	0.71	0.36	38.30	<.0001
DATE(HERB)	3	2.35	0.78	84.37	<.0001
CONC	5	0.11	0.02	2.47	0.035
HERB*CONC	10	0.09	0.009	0.97	0.4754
DATE*CONC(HERB)	15	0.12	0.008	0.89	0.5742

Source	DF	Type III SS	Mean Square	F Value	Pr > F
HERB	2	0.48	0.24	25.66	<.0001
DATE(HERB)	3	2.33	0.78	83.51	<.0001
CONC	5	0.109	0.02	2.34	0.0445
HERB*CONC	10	0.071	0.007	0.76	0.6658
DATE*CONC(HERB)	15	0.12	0.008	0.89	0.5742

Split-Plot Analysis of Matlock Periphytometer experiment using Chlorophyll *b* as dependent variables

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	35	0.50	0.014	18.88	<.0001
Error	141	0.11	0.001		
Corrected Total	176	0.61			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
HERB	2	0.069	0.035	45.88	<.0001
DATE(HERB)	3	0.20	0.068	88.93	<.0001
CONC	5	0.05	0.01	13.25	<.0001
HERB*CONC	10	0.08	0.008	10.38	<.0001
DATE*CONC(HERB)	15	0.10	0.007	8.83	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
HERB	2	0.04	0.02	29.18	<.0001
DATE(HERB)	3	0.21	0.07	90.10	<.0001
CONC	5	0.03	0.006	8.47	<.0001
HERB*CONC	10	0.05	0.005	6.62	<.0001
DATE*CONC(HERB)	15	0.10	0.007	8.83	<.0001

Split-Plot Analysis of Matlock Periphytometer experiment using Chlorophyll *c* as dependent variables

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	35	0.48	0.014	16.10	<.0001
Error	141	0.12	0.0009		
Corrected Total	176	0.60			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
HERB	2	0.05	0.024	27.97	<.0001
DATE(HERB)	3	0.11	0.04	43.55	<.0001
CONC	5	0.05	0.009	10.59	<.0001
HERB*CONC	10	0.14	0.014	15.95	<.0001
DATE*CONC(HERB)	15	0.14	0.009	10.95	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
HERB	2	0.038	0.019	22.27	<.0001
DATE(HERB)	3	0.12	0.039	45.85	<.0001
CONC	5	0.025	0.005	5.87	<.0001
HERB*CONC	10	0.10	0.010	11.11	<.0001
DATE*CONC(HERB)	15	0.14	0.009	10.95	<.0001

Tukey multiple (HSD) comparison of all herbicides and all concentrations of herbicides using ug chlorophyll *a,b,c* and total as the dependent variable. All herbicide concentrations are compared to each other to determine significance.

Effect	HERB	CONC	Estimate	Error	DF	t Value	Pr > |t|
HERB	Aatrex		0.1990	0.1730	3.02	1.15	0.3330
HERB	Glean		0.3524	0.1727	3	2.04	0.1340
HERB	Roundup		0.1610	0.1727	3	0.93	0.4201
CONC		0	0.3085	0.1081	4.12	2.85	0.0447
CONC		1	0.3085	0.1089	4.24	2.83	0.0441
CONC		2	0.2049	0.1100	4.41	1.86	0.1294
CONC		3	0.1882	0.1089	4.23	1.73	0.1549
CONC		4	0.2148	0.1090	4.25	1.97	0.1159
CONC		5	0.1998	0.1085	4.18	1.84	0.1364

Tukey multiple (HSD) comparison of all herbicides and all concentrations of herbicides using ug chlorophyll *a,b,c* and total as the dependent variable. All herbicide concentrations are compared to each other to determine significance. Total Chlorophyll

Effect	HERB CONC	_HERB	_CONC	Estimate	Error	DF	t Value	Pr > |t|	Adjustment	Adj P
HERB*CONC	A 0	A1		0.04574	0.1156	14.6	0.40	0.6980	Tukey-Kramer	1.0000
HERB*CONC	A0	A2		0.04716	0.1237	17.1	0.38	0.7077	Tukey-Kramer	1.0000
HERB*CONC	A0	A3		0.03914	0.1161	14.9	0.34	0.7408	Tukey-Kramer	1.0000
HERB*CONC	A0	A4		0.07875	0.1169	15.3	0.67	0.5104	Tukey-Kramer	1.0000
HERB*CONC	A0	A5		0.07531	0.1156	14.6	0.65	0.5248	Tukey-Kramer	1.0000
HERB*CONC	A1	A2		0.001417	0.1249	17.4	0.01	0.9911	Tukey-Kramer	1.0000
HERB*CONC	A1	A3		-0.00660	0.1178	15.5	-0.06	0.9560	Tukey-Kramer	1.0000
HERB*CONC	A1	A4		0.03301	0.1185	16	0.28	0.7841	Tukey-Kramer	1.0000
HERB*CONC	A1	A5		0.02957	0.1172	15.2	0.25	0.8042	Tukey-Kramer	1.0000
HERB*CONC	A2	A3		-0.00802	0.1255	17.8	-0.06	0.9498	Tukey-Kramer	1.0000
HERB*CONC	A2	A4		0.03160	0.1263	18.4	0.25	0.8052	Tukey-Kramer	1.0000
HERB*CONC	A2	A5		0.02815	0.1249	17.4	0.23	0.8243	Tukey-Kramer	1.0000
HERB*CONC	A3	A4		0.03961	0.1191	16.3	0.33	0.7436	Tukey-Kramer	1.0000
HERB*CONC	A3	A5		0.03617	0.1178	15.5	0.31	0.7628	Tukey-Kramer	1.0000
HERB*CONC	A4	A5		-0.00344	0.1185	16	-0.03	0.9772	Tukey-Kramer	1.0000
HERB*CONC	G0	G1		-0.03857	0.1148	14.3	-0.34	0.7417	Tukey-Kramer	1.0000
HERB*CONC	G0	G2		0.2511	0.1162	14.9	2.16	0.0473	Tukey-Kramer	0.7509
HERB*CONC	G0	G3		0.2735	0.1148	14.3	2.38	0.0315	Tukey-Kramer	0.6265
HERB*CONC	G0	G4		0.2156	0.1138	13.9	1.89	0.0792	Tukey-Kramer	0.8765
HERB*CONC	G0	G5		0.1797	0.1134	13.6	1.58	0.1359	Tukey-Kramer	0.9640
HERB*CONC	G1	G2		0.2897	0.1170	15.3	2.48	0.0254	Tukey-Kramer	0.5725
HERB*CONC	G1	G3		0.3121	0.1156	14.7	2.70	0.0167	Tukey-Kramer	0.4484
HERB*CONC	G1	G4		0.2542	0.1147	14.3	2.22	0.0434	Tukey-Kramer	0.7219
HERB*CONC	G1	G5		0.2182	0.1142	14	1.91	0.0768	Tukey-Kramer	0.8701
HERB*CONC	G2	G3		0.02235	0.1170	15.3	0.19	0.8510	Tukey-Kramer	1.0000
HERB*CONC	G2	G4		-0.03552	0.1161	14.9	-0.31	0.7639	Tukey-Kramer	1.0000
HERB*CONC	G2	G5		-0.07149	0.1156	14.6	-0.62	0.5459	Tukey-Kramer	1.0000
HERB*CONC	G3	G4		-0.05788	0.1147	14.3	-0.50	0.6216	Tukey-Kramer	1.0000
HERB*CONC	G3	G5		-0.09385	0.1142	14	-0.82	0.4251	Tukey-Kramer	1.0000
HERB*CONC	G4	G5		-0.03597	0.1133	13.6	-0.32	0.7558	Tukey-Kramer	1.0000
HERB*CONC	R 0	R 1		-0.00698	0.1149	14.4	-0.06	0.9524	Tukey-Kramer	1.0000
HERB*CONC	R 0	R 2		0.01267	0.1140	13.8	0.11	0.9131	Tukey-Kramer	1.0000
HERB*CONC	R 0	R 3		0.04818	0.1139	13.9	0.42	0.6787	Tukey-Kramer	1.0000
HERB*CONC	R 0	R 4		-0.01311	0.1149	14.4	-0.11	0.9107	Tukey-Kramer	1.0000
HERB*CONC	R 0	R 5		0.07124	0.1129	13.5	0.63	0.5387	Tukey-Kramer	1.0000
HERB*CONC	R 1	R 2		0.01965	0.1181	15.9	0.17	0.8700	Tukey-Kramer	1.0000
HERB*CONC	R 1	R 3		0.05516	0.1180	16	0.47	0.6464	Tukey-Kramer	1.0000
HERB*CONC	R 1	R 4		-0.00613	0.1190	16.6	-0.05	0.9596	Tukey-Kramer	1.0000
HERB*CONC	R 1	R 5		0.07822	0.1171	15.5	0.67	0.5138	Tukey-Kramer	1.0000
HERB*CONC	R 2	R 3		0.03551	0.1171	15.4	0.30	0.7658	Tukey-Kramer	1.0000
HERB*CONC	R 2	R 4		-0.02577	0.1181	15.9	-0.22	0.8301	Tukey-Kramer	1.0000
HERB*CONC	R 2	R 5		0.05858	0.1163	14.9	0.50	0.6217	Tukey-Kramer	1.0000
HERB*CONC	R 3	R 4		-0.06129	0.1180	16	-0.52	0.6106	Tukey-Kramer	1.0000
HERB*CONC	R 3	R 5		0.02306	0.1161	15	0.20	0.8452	Tukey-Kramer	1.0000
HERB*CONC	R 4	R 5		0.08435	0.1171	15.5	0.72	0.4819	Tukey-Kramer	1.0000

Tests of Effect Slices multiple comparison test using ug chl as the dependent variable. This test determines which herbicide at all test concentrations and all concentrations of all herbicides have impacts upon the algae

Effect	HERB	CONC	DF	DF	F Value	Pr > F
HERB*CONC	Aatrex		5	16	0.12	0.9856
HERB*CONC	Glean		5	14.4	2.66	0.0666
HERB*CONC	Roundup		5	15	0.17	0.9704
HERB*CONC		0	2	4.12	0.81	0.5051
HERB*CONC		1	2	4.24	1.11	0.4087
HERB*CONC		2	2	4.4	0.05	0.9548
HERB*CONC		3	2	4.23	0.07	0.9331
HERB*CONC		4	2	4.25	0.10	0.9028
HERB*CONC		5	2	4.18	0.33	0.7335

Tukey multiple (HSD) comparison of all herbicides and all concentrations of herbicides using ug chlorophyll *a,b,c* and total as the dependent variable. All herbicide concentrations are compared to each other to determine significance. Chlorophyll *a*

Effect	HERB	CONC	_HERB	_CONC	Estimate	Error	DF	t Value	Pr > |t|	Adjustment	Adj P
HERB*CONC	A	0	A	1	0.03397	0.04212	156	0.81	0.4212	Tukey-Kramer	1.0000
HERB*CONC	A	0	A	2	0.03525	0.04718	156	0.75	0.4561	Tukey-Kramer	1.0000
HERB*CONC	A	0	A	3	0.03771	0.04324	156	0.87	0.3845	Tukey-Kramer	1.0000
HERB*CONC	A	0	A	4	0.07938	0.04462	156	1.78	0.0772	Tukey-Kramer	0.9460
HERB*CONC	A	0	A	5	0.08417	0.04212	156	2.00	0.0474	Tukey-Kramer	0.8656
HERB*CONC	A	1	A	2	0.001281	0.04746	156	0.03	0.9785	Tukey-Kramer	1.0000
HERB*CONC	A	1	A	3	0.003738	0.04407	156	0.08	0.9325	Tukey-Kramer	1.0000
HERB*CONC	A	1	A	4	0.04541	0.04554	156	1.00	0.3202	Tukey-Kramer	0.9999
HERB*CONC	A	1	A	5	0.05020	0.04289	156	1.17	0.2436	Tukey-Kramer	0.9994
HERB*CONC	A	2	A	3	0.002458	0.04862	156	0.05	0.9597	Tukey-Kramer	1.0000
HERB*CONC	A	2	A	4	0.04413	0.05005	156	0.88	0.3793	Tukey-Kramer	1.0000
HERB*CONC	A	2	A	5	0.04892	0.04746	156	1.03	0.3043	Tukey-Kramer	0.9999
HERB*CONC	A	3	A	4	0.04167	0.04662	156	0.89	0.3727	Tukey-Kramer	1.0000
HERB*CONC	A	3	A	5	0.04646	0.04407	156	1.05	0.2934	Tukey-Kramer	0.9999
HERB*CONC	A	4	A	5	0.004789	0.04554	156	0.11	0.9164	Tukey-Kramer	1.0000
HERB*CONC	G	0	G	1	0.04694	0.04205	156	1.12	0.2660	Tukey-Kramer	0.9997
HERB*CONC	G	0	G	2	0.1187	0.04342	156	2.73	0.0070	Tukey-Kramer	0.3670
HERB*CONC	G	0	G	3	0.1367	0.04205	156	3.25	0.0014	Tukey-Kramer	0.1155
HERB*CONC	G	0	G	4	0.08671	0.04095	156	2.12	0.0358	Tukey-Kramer	0.8020
HERB*CONC	G	0	G	5	0.07341	0.04016	156	1.83	0.0694	Tukey-Kramer	0.9321
HERB*CONC	G	1	G	2	0.07173	0.04409	156	1.63	0.1058	Tukey-Kramer	0.9762
HERB*CONC	G	1	G	3	0.08980	0.04289	156	2.09	0.0379	Tukey-Kramer	0.8156
HERB*CONC	G	1	G	4	0.03977	0.04192	156	0.95	0.3443	Tukey-Kramer	1.0000
HERB*CONC	G	1	G	5	0.02647	0.04107	156	0.64	0.5201	Tukey-Kramer	1.0000
HERB*CONC	G	2	G	3	0.01807	0.04409	156	0.41	0.6825	Tukey-Kramer	1.0000
HERB*CONC	G	2	G	4	-0.03197	0.04321	156	-0.74	0.4605	Tukey-Kramer	1.0000
HERB*CONC	G	2	G	5	-0.04526	0.04234	156	-1.07	0.2867	Tukey-Kramer	0.9998
HERB*CONC	G	3	G	4	-0.05003	0.04192	156	-1.19	0.2345	Tukey-Kramer	0.9993
HERB*CONC	G	3	G	5	-0.06333	0.04107	156	-1.54	0.1251	Tukey-Kramer	0.9860
HERB*CONC	G	4	G	5	-0.01330	0.04004	156	-0.33	0.7403	Tukey-Kramer	1.0000
HERB*CONC	R	0	R	1	-0.03155	0.04250	156	-0.74	0.4590	Tukey-Kramer	1.0000
HERB*CONC	R	0	R	2	0.01068	0.04003	156	0.27	0.7900	Tukey-Kramer	1.0000
HERB*CONC	R	0	R	3	0.02027	0.04100	156	0.49	0.6217	Tukey-Kramer	1.0000
HERB*CONC	R	0	R	4	-0.00543	0.04250	156	-0.13	0.8985	Tukey-Kramer	1.0000
HERB*CONC	R	0	R	5	0.04357	0.03971	156	1.10	0.2742	Tukey-Kramer	0.9998
HERB*CONC	R	1	R	2	0.04223	0.04577	156	0.92	0.3576	Tukey-Kramer	1.0000
HERB*CONC	R	1	R	3	0.05182	0.04662	156	1.11	0.2680	Tukey-Kramer	0.9997
HERB*CONC	R	1	R	4	0.02613	0.04795	156	0.54	0.5867	Tukey-Kramer	1.0000
HERB*CONC	R	1	R	5	0.07512	0.04549	156	1.65	0.1007	Tukey-Kramer	0.9725
HERB*CONC	R	2	R	3	0.009588	0.04421	156	0.22	0.8286	Tukey-Kramer	1.0000
HERB*CONC	R	2	R	4	-0.01611	0.04577	156	-0.35	0.7254	Tukey-Kramer	1.0000
HERB*CONC	R	2	R	5	0.03289	0.04318	156	0.76	0.4474	Tukey-Kramer	1.0000
HERB*CONC	R	3	R	4	-0.02570	0.04662	156	-0.55	0.5823	Tukey-Kramer	1.0000
HERB*CONC	R	3	R	5	0.02330	0.04409	156	0.53	0.5978	Tukey-Kramer	1.0000
HERB*CONC	R	4	R	5	0.04900	0.04549	156	1.08	0.2831	Tukey-Kramer	0.9998

Tests of Effect Slices multiple comparison tests using ug chl as the dependent variable. This test determines which herbicide at all test concentrations and all concentrations of all herbicides have impacts upon the algae

Effect	HERB	CONC	DF	DF	F Value	Pr > F
HERB*CONC	Aatrex		5	156	1.07	0.3796
HERB*CONC	Glean		5	156	2.73	0.0217
HERB*CONC	Roundup		5	156	0.63	0.6785
HERB*CONC		0	2	3.28	0.66	0.5749
HERB*CONC		1	2	3.38	0.29	0.7643
HERB*CONC		2	2	3.43	0.12	0.8892
HERB*CONC		3	2	3.38	0.10	0.9062
HERB*CONC		4	2	3.4	0.28	0.7738
HERB*CONC		5	2	3.32	0.52	0.6344

Tukey multiple (HSD) comparison of all herbicides and all concentrations of herbicides using ug chlorophyll *a,b,c* and total as the dependent variable. All herbicide concentrations are compared to each other to determine significance. Chlorophyll *b*

Effect	HERB	CONC	_HERB	_CONC	Estimate	Error	DF	t Value	Pr > |t|	Adjustment	Adj P
HERB*CONC	A	0	A	1	0.01022	0.03754	14.9	0.27	0.7891	Tukey-Kramer	1.0000
HERB*CONC	A	0	A	2	0.009647	0.03893	16.8	0.25	0.8073	Tukey-Kramer	1.0000
HERB*CONC	A	0	A	3	0.007177	0.03760	15	0.19	0.8512	Tukey-Kramer	1.0000
HERB*CONC	A	0	A	4	0.01235	0.03769	15.2	0.33	0.7477	Tukey-Kramer	1.0000
HERB*CONC	A	0	A	5	0.001405	0.03754	14.9	0.04	0.9706	Tukey-Kramer	1.0000
HERB*CONC	A	1	A	2	-0.00058	0.03915	17.1	-0.01	0.9884	Tukey-Kramer	1.0000
HERB*CONC	A	1	A	3	-0.00305	0.03785	15.4	-0.08	0.9369	Tukey-Kramer	1.0000
HERB*CONC	A	1	A	4	0.002122	0.03794	15.6	0.06	0.9561	Tukey-Kramer	1.0000
HERB*CONC	A	1	A	5	-0.00882	0.03779	15.3	-0.23	0.8186	Tukey-Kramer	1.0000
HERB*CONC	A	2	A	3	-0.00247	0.03922	17.2	-0.06	0.9505	Tukey-Kramer	1.0000
HERB*CONC	A	2	A	4	0.002699	0.03931	17.4	0.07	0.9460	Tukey-Kramer	1.0000
HERB*CONC	A	2	A	5	-0.00824	0.03915	17.1	-0.21	0.8358	Tukey-Kramer	1.0000
HERB*CONC	A	3	A	4	0.005168	0.03801	15.7	0.14	0.8936	Tukey-Kramer	1.0000
HERB*CONC	A	3	A	5	-0.00577	0.03785	15.4	-0.15	0.8808	Tukey-Kramer	1.0000
HERB*CONC	A	4	A	5	-0.01094	0.03794	15.6	-0.29	0.7769	Tukey-Kramer	1.0000
HERB*CONC	G	0	G	1	-0.04285	0.03741	14.7	-1.15	0.2702	Tukey-Kramer	0.9983
HERB*CONC	G	0	G	2	0.05985	0.03760	15	1.59	0.1322	Tukey-Kramer	0.9629
HERB*CONC	G	0	G	3	0.06385	0.03741	14.7	1.71	0.1088	Tukey-Kramer	0.9375
HERB*CONC	G	0	G	4	0.05108	0.03728	14.6	1.37	0.1914	Tukey-Kramer	0.9897
HERB*CONC	G	0	G	5	0.04774	0.03722	14.5	1.28	0.2198	Tukey-Kramer	0.9945
HERB*CONC	G	1	G	2	0.1027	0.03772	15.2	2.72	0.0156	Tukey-Kramer	0.4356
HERB*CONC	G	1	G	3	0.1067	0.03753	14.9	2.84	0.0124	Tukey-Kramer	0.3746
HERB*CONC	G	1	G	4	0.09394	0.03740	14.7	2.51	0.0242	Tukey-Kramer	0.5524
HERB*CONC	G	1	G	5	0.09059	0.03734	14.6	2.43	0.0287	Tukey-Kramer	0.6017
HERB*CONC	G	2	G	3	0.003994	0.03772	15.2	0.11	0.9171	Tukey-Kramer	1.0000
HERB*CONC	G	2	G	4	-0.00877	0.03760	15	-0.23	0.8187	Tukey-Kramer	1.0000
HERB*CONC	G	2	G	5	-0.01211	0.03754	14.9	-0.32	0.7514	Tukey-Kramer	1.0000
HERB*CONC	G	3	G	4	-0.01277	0.03740	14.7	-0.34	0.7377	Tukey-Kramer	1.0000
HERB*CONC	G	3	G	5	-0.01611	0.03734	14.6	-0.43	0.6725	Tukey-Kramer	1.0000
HERB*CONC	G	4	G	5	-0.00334	0.03722	14.5	-0.09	0.9297	Tukey-Kramer	1.0000
HERB*CONC	R	0	R	1	0.000464	0.03742	14.8	0.01	0.9903	Tukey-Kramer	1.0000
HERB*CONC	R	0	R	2	0.002810	0.03733	14.6	0.08	0.9410	Tukey-Kramer	1.0000
HERB*CONC	R	0	R	3	0.01765	0.03729	14.6	0.47	0.6429	Tukey-Kramer	1.0000
HERB*CONC	R	0	R	4	-0.00254	0.03742	14.8	-0.07	0.9469	Tukey-Kramer	1.0000
HERB*CONC	R	0	R	5	0.01421	0.03716	14.4	0.38	0.7077	Tukey-Kramer	1.0000
HERB*CONC	R	1	R	2	0.002346	0.03787	15.5	0.06	0.9514	Tukey-Kramer	1.0000
HERB*CONC	R	1	R	3	0.01719	0.03783	15.4	0.45	0.6559	Tukey-Kramer	1.0000
HERB*CONC	R	1	R	4	-0.00300	0.03795	15.6	-0.08	0.9380	Tukey-Kramer	1.0000
HERB*CONC	R	1	R	5	0.01375	0.03770	15.2	0.36	0.7204	Tukey-Kramer	1.0000
HERB*CONC	R	2	R	3	0.01484	0.03775	15.3	0.39	0.6996	Tukey-Kramer	1.0000
HERB*CONC	R	2	R	4	-0.00535	0.03787	15.5	-0.14	0.8896	Tukey-Kramer	1.0000
HERB*CONC	R	2	R	5	0.01140	0.03762	15.1	0.30	0.7659	Tukey-Kramer	1.0000
HERB*CONC	R	3	R	4	-0.02019	0.03783	15.4	-0.53	0.6011	Tukey-Kramer	1.0000
HERB*CONC	R	3	R	5	-0.00344	0.03758	15	-0.09	0.9283	Tukey-Kramer	1.0000
HERB*CONC	R	4	R	5	0.01675	0.03770	15.2	0.44	0.6631	Tukey-Kramer	1.0000

Tests of Effect Slices multiple comparison tests using ug chl as the dependent variable. This test determines which herbicide at all test concentrations and all concentrations of all herbicides have impacts upon the algae

Effect	HERB	CONC	DF	DF	F Value	Pr > F
HERB*CONC	Aatrex		5	15.8	0.04	0.9992
HERB*CONC	Glean		5	14.8	2.56	0.0732
HERB*CONC	Roundup		5	15.1	0.10	0.9908
HERB*CONC		0	2	6.4	0.63	0.5613
HERB*CONC		1	2	6.57	2.08	0.2002
HERB*CONC		2	2	6.84	0.01	0.9923
HERB*CONC		3	2	6.55	0.09	0.9172
HERB*CONC		4	2	6.57	0.02	0.9814
HERB*CONC		5	2	6.48	0.15	0.8628

Tukey multiple (HSD) comparison of all herbicides and all concentrations of herbicides using ug chlorophyll *a,b,c* and total as the dependent variable. All herbicide concentrations are compared to each other to determine significance. Chlorophyll *c*

Effect	HERB CONC	_HERB_CONC	Estimate	Error	DF	t Value	Pr > |t|	Adjustment	AdjP
HERB*CONC	A 0	A 1	0.004263	0.04427	15	0.10	0.9246	Tukey-Kramer	1.0000
HERB*CONC	A 0	A 2	-0.00088	0.04565	16.6	-0.02	0.9848	Tukey-Kramer	1.0000
HERB*CONC	A 0	A 3	-0.00608	0.04433	15.1	-0.14	0.8927	Tukey-Kramer	1.0000
HERB*CONC	A 0	A 4	-0.00486	0.04442	15.2	-0.11	0.9144	Tukey-Kramer	1.0000
HERB*CONC	A 0	A 5	-0.00583	0.04427	15	-0.13	0.8970	Tukey-Kramer	1.0000
HERB*CONC	A 1	A 2	-0.00515	0.04588	16.9	-0.11	0.9120	Tukey-Kramer	1.0000
HERB*CONC	A 1	A 3	-0.01034	0.04458	15.4	-0.23	0.8196	Tukey-Kramer	1.0000
HERB*CONC	A 1	A 4	-0.00912	0.04466	15.5	-0.20	0.8409	Tukey-Kramer	1.0000
HERB*CONC	A 1	A 5	-0.01009	0.04451	15.3	-0.23	0.8237	Tukey-Kramer	1.0000
HERB*CONC	A 2	A 3	-0.00520	0.04594	17	-0.11	0.9113	Tukey-Kramer	1.0000
HERB*CONC	A 2	A 4	-0.00397	0.04603	17.1	-0.09	0.9322	Tukey-Kramer	1.0000
HERB*CONC	A 2	A 5	-0.00494	0.04588	16.9	-0.11	0.9154	Tukey-Kramer	1.0000
HERB*CONC	A3	A 4	0.001223	0.04472	15.6	0.03	0.9785	Tukey-Kramer	1.0000
HERB*CONC	A 3	A 5	0.000251	0.04458	15.4	0.01	0.9956	Tukey-Kramer	1.0000
HERB*CONC	A 4	A 5	-0.00097	0.04466	15.5	-0.02	0.9829	Tukey-Kramer	1.0000
HERB*CONC	G 0	G1	-0.04478	0.04414	14.8	-1.01	0.3266	Tukey-Kramer	0.9996
HERB*CONC	G 0	G2	0.07173	0.04433	15.1	1.62	0.1264	Tukey-Kramer	0.9579
HERB*CONC	G 0	G3	0.07584	0.04414	14.8	1.72	0.1066	Tukey-Kramer	0.9346
HERB*CONC	G 0	G4	0.07658	0.04402	14.7	1.74	0.1029	Tukey-Kramer	0.9287
HERB*CONC	G 0	G5	0.05901	0.04396	14.6	1.34	0.2000	Tukey-Kramer	0.9915
HERB*CONC	G 1	G 2	0.1165	0.04445	15.2	2.62	0.0191	Tukey-Kramer	0.4904
HERB*CONC	G 1	G 3	0.1206	0.04426	15	2.73	0.0157	Tukey-Kramer	0.4341
HERB*CONC	G 1	G 4	0.1214	0.04414	14.8	2.75	0.0150	Tukey-Kramer	0.4216
HERB*CONC	G 1	G 5	0.1038	0.04408	14.7	2.35	0.0329	Tukey-Kramer	0.6431
HERB*CONC	G 2	G 3	0.00412	0.04445	15.2	0.09	0.9274	Tukey-Kramer	1.0000
HERB*CONC	G 2	G 4	0.00485	0.04433	15.1	0.11	0.9143	Tukey-Kramer	1.0000
HERB*CONC	G 2	G 5	-0.01272	0.04427	15	-0.29	0.7778	Tukey-Kramer	1.0000
HERB*CONC	G 3	G4	0.00073	0.04414	14.8	0.02	0.9870	Tukey-Kramer	1.0000
HERB*CONC	G 3	G 5	-0.0168	0.04408	14.7	-0.38	0.7080	Tukey-Kramer	1.0000
HERB*CONC	G 4	G 5	-0.0176	0.04396	14.6	-0.40	0.6952	Tukey-Kramer	1.0000
HERB*CONC	R 0	R 1	0.0242	0.04415	14.8	0.55	0.5921	Tukey-Kramer	1.0000
HERB*CONC	R 0	R 2	-0.0014	0.04407	14.7	-0.03	0.9751	Tukey-Kramer	1.0000
HERB*CONC	R 0	R 3	0.00894	0.04403	14.7	0.20	0.8418	Tukey-Kramer	1.0000
HERB*CONC	R 0	R 4	-0.0050	0.04415	14.8	-0.11	0.9123	Tukey-Kramer	1.0000
HERB*CONC	R 0	R 5	0.0137	0.04391	14.5	0.31	0.7590	Tukey-Kramer	1.0000
HERB*CONC	R 1	R 2	-0.0256	0.04459	15.4	-0.57	0.5745	Tukey-Kramer	1.0000
HERB*CONC	R 1	R 3	-0.0152	0.04455	15.4	-0.34	0.7370	Tukey-Kramer	1.0000
HERB*CONC	R 1	R 4	-0.0291	0.04467	15.5	-0.65	0.5239	Tukey-Kramer	1.0000
HERB*CONC	R 1	R 5	-0.0105	0.04443	15.2	-0.24	0.8172	Tukey-Kramer	1.0000
HERB*CONC	R 2	R 3	0.0103	0.04447	15.3	0.23	0.8192	Tukey-Kramer	1.0000
HERB*CONC	R 2	R 4	-0.0036	0.04459	15.4	-0.08	0.9376	Tukey-Kramer	1.0000
HERB*CONC	R 2	R 5	0.0151	0.04435	15.1	0.34	0.7378	Tukey-Kramer	1.0000
HERB*CONC	R 3	R 4	-0.0139	0.04455	15.4	-0.31	0.7594	Tukey-Kramer	1.0000
HERB*CONC	R 3	R 5	0.00479	0.04430	15.1	0.11	0.9154	Tukey-Kramer	1.0000
HERB*CONC	R 4	R 5	0.0187	0.04443	15.2	0.42	0.6801	Tukey-Kramer	1.0000

Tests of Effect Slices multiple comparison tests using ug chl as the dependent variable. This test determines which herbicide at all test concentrations and all concentrations of all herbicides have impacts upon the algae

Effect	HERB	CONC	Num DF	Den DF	F Value	Pr > F
Herb*Conc	Aatrex		5	15.7	0.02	0.9999
Herb*Conc	Glean		5	14.9	2.61	0.0687
Herb*Conc	Roundup		5	15.1	0.12	0.9853
Herb*Conc		0	2	11.1	1.12	0.3606
Herb*Conc		1	2	11.5	3.70	0.0574
Herb*Conc		2	2	12.1	0.03	0.9726
Herb*Conc		3	2	11.5	0.01	0.9936
Herb*Conc		4	2	11.5	0.06	0.9462
Herb*Conc		5	2	11.3	0.06	0.9442

APPENDIX C

DATA USED TO CALCULATE THE NUMBER OF PERIPHYTOMETERS NEEDED

Total Variance Calculations used to determine S in Matlock Periphytometer Equation

	Artificial Substrate	Natural Substrate	Reference
	33	58	Brown 1976
	193	194	Burkholder and Wetzel,1989
Total	226	252	
STDDEV	113.14	96.17	
Mean	113	126	
These studies used total number of taxa present			
	6.96	5.65	Cattameo and Kalff, 1978
	0.5	4.96	Loeb, 1981
	10	8.8	Fontaine and Nigh, 1983
Total	17.46	19.41	
STDDEV	4.85	2.05	
Mean	5.82	6.47	
These studies used differences in Chl a concentrations.			

	Substrate	%Similarity	Lowe and Gale, 1980
	Ver.Slate Vs Stone	78	
	Sandy Slate vs Stone	78	
	Frosted glass vs stone	67	
	smooth glass vs stone	67	
	Acrylic vs stone	60	
Mean		70	
STDDEV		7.84	

APPENDIX D

RAW DATA FIELD

Raw data from Matlock Periphytometers. Chlorophyll values in ug cholorphyll/cm^2 Concentration values in amount of stock solution added to the Matlock Periphytometer.

Obs	HERB	DATE	TRT	CONC	CHLA	CHLB	CHLC	TOTCHL
1	Roundup	09/12/02	Control	0	0.218	0.018	0.027	0.263
2	Roundup	09/12/02	Control	0	0.090	0.033	0.029	0.152
3	Roundup	09/12/02	Control	0	0.203	0.064	0.030	0.296
4	Roundup	09/12/02	Control	0	0.231	0.061	0.076	0.368
5	Roundup	09/12/02	Control	0	0.147	0.016	0.017	0.180
6	Roundup	09/12/02	Control	0	0.211	0.054	0.051	0.316
7	Roundup	09/12/02	Control	0	0.117	0.044	0.035	0.196
8	Roundup	10/03/02	Control	0	0.074	0.021	0.022	0.117
9	Roundup	10/03/02	Control	0	0.054	0.011	0.014	0.079
10	Roundup	10/03/02	Control	0	0.148	0.021	0.030	0.199
11	Roundup	10/03/02	Control	0	0.052	0.001	0.008	0.061
12	Roundup	10/03/02	Control	0	0.049	0.008	0.013	0.070
13	Roundup	10/03/02	Control	0	0.108	0.015	0.021	0.143
14	Roundup	10/03/02	Control	0	0.056	0.007	0.011	0.075
15	Roundup	09/12/02	1ml	1	0.196	0.025	0.016	0.237
16	Roundup	09/12/02	1ml	1	0.101	0.007	0.007	0.115
17	Roundup	09/12/02	1ml	1	0.100	0.005	0.005	0.110
18	Roundup	09/12/02	1ml	1	0.081	0.015	0.018	0.114
19	Roundup	10/03/02	1ml	1	0.088	0.017	0.026	0.131
20	Roundup	10/03/02	1ml	1	-0.005	0.021	0.018	0.034
21	Roundup	10/03/02	1ml	1	0.184	0.024	0.036	0.244
22	Roundup	10/03/02	1ml	1	0.512	0.096	-0.100	0.508
23	Roundup	09/12/02	2ml	2	0.090	0.016	0.021	0.127
24	Roundup	09/12/02	2ml	2	0.126	0.015	0.010	0.152
25	Roundup	09/12/02	2ml	2	0.143	0.030	0.021	0.194
26	Roundup	10/03/02	2ml	2	0.101	0.012	0.020	0.133
27	Roundup	10/03/02	2ml	2	0.078	0.019	0.032	0.129
28	Roundup	10/03/02	2ml	2	0.022	0.009	0.011	0.042
29	Roundup	10/03/02	2ml	2	0.075	0.025	0.041	0.141
30	Roundup	10/03/02	2ml	2	0.256	0.019	0.043	0.318
31	Roundup	10/03/02	2ml	2	0.085	0.087	0.103	0.275
32	Roundup	10/03/02	2ml	2	0.114	0.017	0.025	0.156
33	Roundup	09/12/02	3ml	3	0.111	0.011	0.006	0.129
34	Roundup	09/12/02	3ml	3	0.125	0.009	0.013	0.147
35	Roundup	09/12/02	3ml	3	0.070	0.007	0.006	0.083
36	Roundup	09/12/02	3ml	3	0.070	0.010	0.005	0.084
37	Roundup	10/03/02	3ml	3	0.091	0.011	0.020	0.122
38	Roundup	10/03/02	3ml	3	0.066	0.011	0.017	0.095
39	Roundup	10/03/02	3ml	3	0.155	0.027	0.054	0.236
40	Roundup	10/03/02	3ml	3	0.058	-0.022	0.025	0.062
41	Roundup	10/03/02	3ml	3	0.187	0.017	0.030	0.234
42	Roundup	09/12/02	4ml	4	0.120	0.027	0.003	0.149
43	Roundup	09/12/02	4ml	4	0.215	0.123	0.147	0.485
44	Roundup	09/12/02	4ml	4	0.176	0.017	0.019	0.212
45	Roundup	09/12/02	4ml	4	0.214	0.029	0.037	0.280
46	Roundup	10/03/02	4ml	4	0.121	0.016	0.026	0.164
47	Roundup	10/03/02	4ml	4	0.130	-0.006	0.004	0.128
48	Roundup	10/03/02	4ml	4	0.037	0.011	0.005	0.054
49	Roundup	10/03/02	4ml	4	0.035	0.017	0.018	0.070
50	Roundup	09/12/02	5ml	5	0.224	0.020	0.023	0.268
51	Roundup	09/12/02	5ml	5	0.090	0.014	0.011	0.116

Obs	HERB	DATE	TRT	CONC	CHLA	CHLB	CHLC	TOTCHL
52	Roundup	09/12/02	5ml	5	0.051	0.007	0.005	0.063
53	Roundup	09/12/02	5ml	5	0.030	0.011	0.003	0.044
54	Roundup	09/12/02	5ml	5	0.060	0.013	0.010	0.083
55	Roundup	10/03/02	5ml	5	0.064	0.014	0.024	0.102
56	Roundup	10/03/02	5ml	5	0.039	0.010	0.012	0.061
57	Roundup	10/03/02	5ml	5	0.101	0.012	0.017	0.130
58	Roundup	10/03/02	5ml	5	0.041	0.009	0.012	0.062
59	Roundup	10/03/02	5ml	5	0.120	0.015	0.020	0.155
60	Aatrex	6/25/2002	Control	0	0.339	0.034	0.025	0.399
61	Aatrex	6/25/2002	Control	0	0.390	0.054	0.030	0.474
62	Aatrex	6/25/2002	Control	0	0.284	0.030	0.030	0.344
63	Aatrex	6/25/2002	Control	0	0.318	0.041	0.029	0.388
64	Aatrex	6/25/2002	Control	0	0.199	0.022	0.021	0.241
65	Aatrex	6/25/2002	Control	0	0.306	0.035	0.024	0.366
66	Aatrex	10/11/200	Control	0	0.054	0.043	-0.001	0.095
67	Aatrex	10/11/200	Control	0	0.081	0.044	0.004	0.129
68	Aatrex	10/11/200	Control	0	0.107	0.049	0.008	0.163
69	Aatrex	10/11/200	Control	0	0.088	0.044	0.001	0.133
70	Aatrex	10/11/200	Control	0	0.041	0.044	0.009	0.095
71	Aatrex	6/25/2002	1ml	1	0.397	0.023	0.036	0.456
72	Aatrex	6/25/2002	1ml	1	0.098	0.011	0.015	0.123
73	Aatrex	6/25/2002	1ml	1	0.165	0.015	0.013	0.193
74	Aatrex	6/25/2002	1ml	1	0.146	0.015	0.016	0.176
75	Aatrex	6/25/2002	1ml	1	0.276	0.023	0.021	0.320
76	Aatrex	6/25/2002	1ml	1	0.271	0.007	0.029	0.307
77	Aatrex	6/25/2002	1ml	1	0.367	0.031	0.039	0.437
78	Aatrex	10/11/200	1ml	1	0.054	0.037	-0.007	0.085
79	Aatrex	10/11/200	1ml	1	0.045	0.041	-0.002	0.084
80	Aatrex	10/11/200	1ml	1	0.122	0.051	0.002	0.175
81	Aatrex	6/25/2002	2ml	2	0.338	0.025	0.032	0.396
82	Aatrex	6/25/2002	2ml	2	0.177	0.014	0.020	0.211
83	Aatrex	6/25/2002	2ml	2	0.065	0.007	0.009	0.080
84	Aatrex	6/25/2002	2ml	2	0.313	0.017	0.029	0.359
85	Aatrex	6/25/2002	2ml	2	0.194	0.012	0.023	0.229
86	Aatrex	6/25/2002	2ml	2	0.416	0.048	0.056	0.519
87	Aatrex	10/11/200	2ml	2	0.042	0.042	0.003	0.086
88	Aatrex	6/25/2002	3ml	3	0.305	0.025	0.039	0.370
89	Aatrex	6/25/2002	3ml	3	0.247	0.024	0.033	0.304
90	Aatrex	6/25/2002	3ml	3	0.082	0.013	0.015	0.110
91	Aatrex	6/25/2002	3ml	3	0.261	0.017	0.031	0.310
92	Aatrex	6/25/2002	3ml	3	0.246	0.027	0.032	0.306
93	Aatrex	6/25/2002	3ml	3	0.361	0.034	0.061	0.456
94	Aatrex	10/11/200	3ml	3	0.026	0.046	0.010	0.081
95	Aatrex	10/11/200	3ml	3	0.104	0.044	0.011	0.158
96	Aatrex	10/11/200	3ml	3	0.028	0.040	0.001	0.068
97	Aatrex	6/25/2002	4ml	4	0.137	0.012	0.023	0.172
98	Aatrex	6/25/2002	4ml	4	0.184	0.023	0.039	0.247
99	Aatrex	6/25/2002	4ml	4	0.258	0.037	0.028	0.323
100	Aatrex	6/25/2002	4ml	4	0.234	0.009	0.032	0.276
101	Aatrex	6/25/2002	4ml	4	0.065	0.012	0.014	0.091
102	Aatrex	10/11/200	4ml	4	0.132	0.012	0.017	0.162
103	Aatrex	10/11/200	4ml	4	0.036	0.044	0.003	0.084
104	Aatrex	10/11/200	4ml	4	0.037	0.057	0.020	0.114
105	Aatrex	6/25/2002	5ml	5	0.091	0.009	0.014	0.113
106	Aatrex	6/25/2002	5ml	5	0.330	0.043	0.035	0.408

Obs	HERB	DATE	TRT	CONC	CHLA	CHLB	CHLC	TOTCHL
107	Aatrex	6/25/2002	5ml	5	0.182	0.028	0.031	0.241
108	Aatrex	6/25/2002	5ml	5	0.182	0.035	0.021	0.238
109	Aatrex	6/25/2002	5ml	5	0.215	0.033	0.033	0.281
110	Aatrex	6/25/2002	5ml	5	0.127	0.026	0.021	0.174
111	Aatrex	6/25/2002	5ml	5	0.211	0.039	0.053	0.303
112	Aatrex	10/11/200	5ml	5	0.026	0.040	0.005	0.071
113	Aatrex	10/11/200	5ml	5	0.037	0.054	0.021	0.112
114	Aatrex	10/11/200	5ml	5	0.038	0.049	0.012	0.098
115	Glean	8/22/2002	Control	0	0.576	0.117	0.107	0.799
116	Glean	8/22/2002	Control	0	0.245	0.069	0.035	0.348
117	Glean	8/22/2002	Control	0	0.434	0.096	0.074	0.604
118	Glean	8/22/2002	Control	0	0.600	0.289	0.310	1.199
119	Glean	8/22/2002	Control	0	0.750	0.293	0.297	1.340
120	Glean	10/19/2002	Control	0	0.080	0.012	0.031	0.122
121	Glean	10/19/2002	Control	0	0.029	-0.010	-0.009	0.010
122	Glean	10/19/2002	Control	0	0.200	0.010	0.018	0.228
123	Glean	10/19/2002	Control	0	0.050	0.002	0.008	0.060
124	Glean	10/19/2002	Control	0	0.112	-0.001	0.007	0.119
125	Glean	10/19/2002	Control	0	0.244	0.029	0.054	0.327
126	Glean	8/22/2002	1ml	1	0.315	0.228	0.243	0.786
127	Glean	8/22/2002	1ml	1	0.308	0.228	0.249	0.784
128	Glean	8/22/2002	1ml	1	0.541	0.278	0.266	1.084
129	Glean	8/22/2002	1ml	1	0.573	0.288	0.277	1.137
130	Glean	8/22/2002	1ml	1	0.700	0.329	0.316	1.346
131	Glean	8/22/2002	1ml	1	0.543	0.252	0.279	1.073
132	Glean	10/19/2002	1ml	1	0.044	-0.008	-0.007	0.028
133	Glean	10/19/2002	1ml	1	0.012	-0.003	-0.003	0.007
134	Glean	10/19/2002	1ml	1	0.011	-0.011	-0.007	-0.006
135	Glean	10/19/2002	1ml	1	0.028	-0.002	0.002	0.029
136	Glean	8/22/2002	2ml	2	0.411	0.044	0.031	0.485
137	Glean	8/22/2002	2ml	2	0.312	0.030	0.021	0.363
138	Glean	8/22/2002	2ml	2	0.288	0.050	0.025	0.362
139	Glean	8/22/2002	2ml	2	0.327	0.099	0.025	0.451
140	Glean	8/22/2002	2ml	2	0.435	0.048	0.046	0.529
141	Glean	8/22/2002	2ml	2	0.453	0.081	0.049	0.583
142	Glean	10/19/2002	2ml	2	0.041	0.003	0.011	0.055
143	Glean	10/19/2002	2ml	2	0.050	0.003	0.001	0.054
144	Glean	10/19/2002	2ml	2	0.022	0.003	0.010	0.035
145	Glean	8/22/2002	3ml	3	0.417	0.079	0.037	0.532
146	Glean	8/22/2002	3ml	3	0.141	0.013	0.000	0.154
147	Glean	8/22/2002	3ml	3	0.179	0.015	0.005	0.199
148	Glean	8/22/2002	3ml	3	0.468	0.069	0.042	0.579
149	Glean	8/22/2002	3ml	3	0.322	0.040	0.021	0.383
150	Glean	8/22/2002	3ml	3	0.465	0.083	0.034	0.582
151	Glean	10/19/2002	3ml	3	0.063	0.012	0.022	0.097
152	Glean	10/19/2002	3ml	3	0.011	-0.007	-0.008	-0.004
153	Glean	10/19/2002	3ml	3	0.073	0.010	0.019	0.103
154	Glean	10/19/2002	3ml	3	0.038	-0.003	0.000	0.035
155	Glean	8/22/2002	4ml	4	0.462	0.102	0.035	0.599
156	Glean	8/22/2002	4ml	4	0.652	0.137	-0.003	0.785
157	Glean	8/22/2002	4ml	4	0.240	0.041	0.015	0.296
158	Glean	8/22/2002	4ml	4	0.312	0.047	0.025	0.384
159	Glean	8/22/2002	4ml	4	0.363	0.064	0.031	0.458
160	Glean	8/22/2002	4ml	4	0.445	0.080	0.042	0.566
161	Glean	10/19/2002	4ml	4	0.019	-0.003	-0.001	0.015

Obs	HERB	DATE	TRT	CONC	CHLA	CHLB	CHLC	TOTCHL
162	Glean	10/19/2002	4ml	4	0.028	-0.010	-0.005	0.013
163	Glean	10/19/2002	4ml	4	0.065	0.008	0.023	0.095
164	Glean	10/19/2002	4ml	4	0.076	0.004	0.010	0.090
165	Glean	10/19/2002	4ml	4	0.066	-0.005	-0.001	0.060
166	Glean	8/22/2002	5ml	5	0.313	0.081	0.029	0.424
167	Glean	8/22/2002	5ml	5	0.195	0.040	0.026	0.261
168	Glean	8/22/2002	5ml	5	0.543	0.071	0.067	0.682
169	Glean	8/22/2002	5ml	5	0.328	0.047	0.042	0.416
170	Glean	8/22/2002	5ml	5	0.324	0.057	0.046	0.427
171	Glean	8/22/2002	5ml	5	0.478	0.109	0.063	0.650
172	Glean	8/22/2002	5ml	5	0.724	0.151	0.093	0.968
173	Glean	10/19/2002	5ml	5	0.123	0.013	0.020	0.156
174	Glean	10/19/2002	5ml	5	0.089	0.009	0.018	0.116
175	Glean	10/19/2002	5ml	5	0.052	0.001	0.008	0.061
176	Glean	10/19/2002	5ml	5	0.078	0.001	0.007	0.086
177	Glean	10/19/2002	5ml	5	0.053	0.000	0.008	0.062

APPENDIX E

RAW DATA BOTTLE TESTS

Bottle test results for *P. oedogonia* with growth rates as mg fresh weight per day and chlorophyll *a* as mg chl *a*/mg fresh weight.

Date of test	Amount of stock solution ml/flask	Growth Rate mg fresh weight per day	mg chl/mg Fresh weight
Aatrex			
8/22	0	0.058	0.642
8/22	0	0.179	0.530
8/22	0	0.037	0.554
8/22	0	0.137	0.444
8/22	0	0.258	0.458
9/1	0	0.241	1.291
9/1	0	0.383	1.304
9/1	0	0.304	1.325
9/1	0	0.284	1.537
12/16	0	0.175	1.067
12/16	0	0.157	1.424
12/16	0	0.194	1.336
12/16	0	0.157	1.157
12/16	0	0.251	1.437
12/16	0	0.315	1.220
8/22	1	0.000	0.831
8/22	1	0.000	0.856
8/22	1	0.000	0.547
9/1	1	0.014	1.703
9/1	1	0.270	1.307
9/1	1	0.000	2.061
12/16	1	0.000	1.852
12/16	1	0.000	1.895
12/16	1	0.000	1.969
12/16	1	0.000	1.960
8/22	2	0.000	0.673
8/22	2	0.000	0.997
8/22	2	0.000	0.883
9/1	2	0.000	2.062
9/1	2	0.000	0.846
9/1	2	0.092	1.801
12/16	2	0.000	1.904
12/16	2	0.000	1.590
12/16	2	0.000	2.032
12/16	2	0.000	2.221
8/22	3	0.000	0.731
8/22	3	0.000	0.764
8/22	3	0.000	0.724
9/1	3	0.000	1.789
9/1	3	0.024	1.314
9/1	3	0.028	1.446
12/16	3	0.000	2.237
12/16	3	0.000	2.094

Date	Amount of stock solution	Growth Rate	mg chl/mg Fresh weight
12/16	3	0.000	1.996
12/16	3	0.000	2.020
8/22	4	0.037	0.593
8/22	4	0.000	0.493
8/22	4	0.000	0.453
9/1	4	0.000	1.934
9/1	4	0.013	1.674
9/1	4	0.013	1.923
12/16	4	0.000	1.878
12/16	4	0.000	1.849
12/16	4	0.026	1.928
12/16	4	0.000	1.595
8/22	5	0.000	0.565
8/22	5	0.000	0.408
8/22	5	0.000	0.315
9/1	5	0.017	1.692
9/1	5	0.000	1.511
9/1	5	0.015	1.275
12/16	5	0.000	1.446
12/16	5	0.000	1.905
12/16	5	0.000	2.175
12/16	5	0.000	2.812
Roundup			
10/20	0	0.377	0.845
10/20	0	0.339	0.874
10/20	0	0.202	1.107
10/20	0	0.337	0.771
12/16	0	0.175	1.067
12/16	0	0.157	1.424
12/16	0	0.194	1.336
12/16	0	0.157	1.157
12/16	0	0.251	1.437
12/16	0	0.315	1.220
10/20	1	0.263	0.859
10/20	1	0.436	0.737
10/20	1	0.312	0.704
10/20	1	0.385	0.654
12/16	1	0.297	1.297
12/16	1	0.304	0.988
12/16	1	0.119	1.282
12/16	1	0.258	1.159
10/20	2	0.218	0.763
10/20	2	0.312	0.919
10/20	2	0.227	0.755
10/20	2	0.323	0.815
12/16	2	0.241	1.502
12/16	2	0.218	1.273
12/16	2	0.014	1.409
12/16	2	0.256	1.078
10/20	3	0.000	0.832

Date	Amount of stock solution	Growth rate	mg chl/mg Fresh weight
10/20	3	0.157	0.838
10/20	3	0.312	0.549
10/20	3	0.249	0.783
12/16	3	0.000	1.272
12/16	3	0.311	1.180
12/16	3	0.000	0.853
12/16	3	0.000	1.615
10/20	4	0.147	0.561
10/20	4	0.000	0.868
10/20	4	0.309	0.600
10/20	4	0.256	0.708
12/16	4	0.000	0.912
12/16	4	0.000	1.972
12/16	4	0.125	1.314
12/16	4	0.230	1.611
10/20	5	0.000	0.866
10/20	5	0.037	0.593
10/20	5	0.256	0.585
10/20	5	0.224	0.847
12/16	5	0.157	1.757
12/16	5	0.292	1.597
12/16	5	0.000	1.062
12/16	5	0.187	1.542
Glean			
10/27	0	0.377	0.845
10/27	0	0.339	0.874
10/27	0	0.202	1.107
10/27	0	0.337	0.771
12/22	0	0.363	0.899
12/22	0	0.194	0.927
12/22	0	0.272	0.961
12/22	0	0.202	1.211
12/22	0	0.284	0.846
12/22	0	0.274	0.775
12/22	0	0.157	1.079
12/22	0	0.092	1.208
12/22	0	0.328	0.844
12/22	0	0.157	1.427
10/27	1	0.000	0.692
10/27	1	0.000	0.649
10/27	1	0.218	0.841
10/27	1	0.270	0.982
12/22	1	0.058	1.114
12/22	1	0.125	0.550
12/22	1	0.113	0.846
12/22	1	0.000	0.956
10/27	2	0.000	0.737
10/27	2	0.000	0.971
10/27	2	0.000	0.926
10/27	2	0.157	0.895

Date test	Amount of stock added	Growth Rate	mg chl/mg Fresh weight
12/22	2	0.092	1.387
12/22	2	0.000	1.673
12/22	2	0.026	1.611
12/22	2	0.058	1.150
10/27	3	0.000	0.801
10/27	3	0.000	0.759
10/27	3	0.000	0.811
10/27	3	0.000	0.653
12/22	3	0.048	1.168
12/22	3	0.000	1.035
12/22	3	0.106	0.663
12/22	3	0.000	0.662
10/27	4	0.137	1.423
10/27	4	0.000	1.113
10/27	4	0.106	0.839
10/27	4	0.000	0.830
12/22	4	0.058	0.446
12/22	4	0.000	3.133
12/22	4	0.142	1.000
12/22	4	0.076	1.474
10/27	5	0.000	1.017
10/27	5	0.000	1.039
10/27	5	0.026	1.178
10/27	5	0.000	1.046
12/22	5	0.014	2.132
12/22	5	0.000	0.697
12/22	5	0.000	0.923
12/22	5	0.000	0.994
Surfactant	0	0.363	0.899
	0	0.194	0.927
	0	0.272	0.961
	0	0.202	1.211
	0	0.284	0.846
	0	0.274	0.775
	0	0.157	1.079
	0	0.092	1.208
	0	0.328	0.844
	0	0.157	1.427
	1	0.106	1.301
	1	0.191	1.016
	1	0.208	0.923
	1	0.000	0.853
	2	0.026	0.954
	2	0.136	0.804
	2	0.000	0.780
	2	0.000	0.835
	3	0.000	0.783
	3	0.000	0.853
	3	0.152	0.749
	3	0.026	0.582

	Amount of stock added	Growth Rate	mg chl/mg Fresh weight
	4	0.105	1.044
	4	0.084	1.049
	4	0.000	1.087
	4	0.000	1.537
	5	0.000	1.551
	5	0.048	1.227
	5	0.000	1.274
	5	0.026	1.185

Bottle test results for *P. oedogonia* with growth rates as mg fresh weight per day and chlorophyll *a* as mg chl *a*/mg fresh weight.

Active ingredients		Growth Rate	mg chl/mg Fresh weight
Atrazine			
	Control	0.366	1.391
		0.379	0.669
		0.330	0.779
		0.171	0.638
	100	0.000	0.857
		0.076	0.766
		0.147	0.759
		0.137	0.769
	75	0.000	1.086
		0.037	0.982
		0.320	0.744
		-0.230	0.783
	50	0.000	1.084
		0.198	0.784
		0.288	0.840
		0.293	0.701
	25	0.278	1.009
		0.119	0.753
		0.333	1.005
		0.325	1.125
Glean			
	Control	0.292	0.911
		0.312	0.870
		0.297	0.904
		0.344	0.842
	0.5 mg	0.341	0.767
		0.307	0.858
		0.284	0.841
		0.249	0.914
	5.0 mg	0.244	0.986
		0.215	0.871
		0.364	0.707
		0.119	0.871
	50	0.171	0.853
		0.076	0.770

		Growth Rate	mg chl/mg Fresh weight
		0.000	0.976
		0.058	0.743
Roundup			
	Control	0.191	0.862
		0.293	0.802
		0.212	0.896
		0.152	1.246
	100 mg	0.000	1.052
		-0.099	0.843
		-0.051	0.617
		0.000	0.925
	500 mg	0.092	0.996
		0.067	0.932
		0.048	0.968
		-0.230	0.633
	1000 mg	0.000	0.660
		0.000	1.340
		0.000	1.758
		0.000	0.897
Mixture			
	Control	0.340	0.874
	Control	0.312	1.091
	Control	0.339	1.032
	Control	0.249	0.989
	Control	0.272	1.025
	Control	0.320	1.091
	Atr/Chlor	0.254	1.371
	Atr/Chlor	0.157	1.511
	Atr/Chlor	0.166	1.210
	Atr/Chlor	0.000	1.239
	Atr/Chlor	0.131	1.231
	Atr/Chlor	0.000	1.138
	Atr/Chlor	0.000	1.262
	Atr/Chlor	-0.032	1.312
	Atr/Glyph	0.014	1.173
	Atr/Glyph	0.026	1.179
	Atr/Glyph	0.205	1.216
	Atr/Glyph	0.000	1.412
	Atr/Glyph	0.000	1.037
	Atr/Glyph	0.000	1.560
	Atr/Glyph	0.092	1.384
	Atr/Glyph	0.037	1.340
	Chlor/Glyp	0.000	1.147
	Chlor/Glyp	0.000	1.120
	Chlor/Glyp	0.000	1.052
	Chlor/Glyp	0.000	1.028
	Chlor/Glyp	0.000	1.044
	Chlor/Glyp	0.000	1.121
	Chlor/Glyp	0.137	0.741
	Chlor/Glyp	0.000	0.929

VITA (2)

PETER ARTHUR KISH

CANDIDATE FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

Thesis: Effects of Roundup, Glean, Aatrex and Their Active Ingredients (Glyphosate, Chlorsulfuron and Atrazine) on Periphyton Communities Studied by using Matlock Periphytometer and Bottle Tests

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