CHARACTERIZATION OF THE ACTIVITY OF FLUAZIFOP-BUTYL ON BRISTLY STARBUR (*ACANTHOSPERMUM HISPIDUM DC*.) AND TRIMETHYLSULFONIUM SALT OF GLYPHOSATE ON ROUND-UP READY COTTON™ (*GOSSYPIUM HIRSUTUM L*.)

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL
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I dedicate this thesis to my loving family.

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Fluazifop-p-butyl is a herbicide registered for grassy weed control in several agronomic and horticultural crops. This herbicide is thought to be specific for grass weed control, but observational evidence suggests the broadleaf weed, bristly starbur, is affected by fluazifop. Furthermore, an alternative mode of action may occur in this species. To further characterize fluazifop activity on bristly starbur, ion leakage and chlorophyll fluorescence studies were performed.

There was differential response of fluazifop rate under light and dark conditions, with greater ion leakage in the dark. Ion leakage caused by fluazifop was also compared to compounds with known mechanisms of action. Fluazifop behaved most similarly to paraquat under light conditions, with complete ion leakage observed after 24 and 96 hours for paraquat and fluazifop, respectively. In contrast, nearly total ion leakage by fluazifop occurred after only 24 hours under dark conditions, behaving most similarly to

2,4 DNP and gramicidin. Fluazifop impacted chlorophyll fluorescence, behaving similarly to the photosynthetic inhibitors diuron and paraquat. Although this suggests the mechanism is photosynthetic activity in the dark suggests some level of membrane activity. Collectively results indicate a more direct impact possibly through membrane uncoupling, which would also explain high fluorescence.

Glyphosate is a broad spectrum herbicide that is used in wide variety of cropping systems, including, Round-up-Ready™ crops. At the time of Round-up-Ready™ cotton registration, two formulations of glyphosate were available--the isopropylamine salt and the trimethylsulfonium salt. Similar toxicities and weed control have been reported with both formulations, but several studies have demonstrated injury to Round-up-Ready™ cotton with the TMS formulation and suggested that the trimesium salt itself was phytotoxic to cotton. Studies were conducted on ion leakage and chlorophyll 'a' florescence to further characterize the activity of trimethylsulfonium salt of glyphosate on Round-up-Ready™ cotton. There was differential response of trimethylsulfonium salt of glyphosate under light and dark conditions, with greater ion leakage in the light. The effect of TMS formulation was markedly reduced in the dark, acting similarly to diuron and paraquat. This correlates with compounds that only inhibit photosynthesis. Chlorophyll luorescence studies were also performed and the trimethylsulfonium salt of glyphosate increased in chlorophyll 'a' fluorescence, suggesting a photosynthetically active compound.

CHAPTER 1
LITERATURE REVIEW

Bristly Starbur and Fluazifop-Butyl

Bristly starbur (*Acanthospermum hispidum DC*.) is an annual, non-native weed from central and South America (Vester 1974). Bristly starbur is weedy in it's native range, found throughout Central America to southward Argentina in South America (Hall et al. 1991). It is categorized as one of the main weeds in many crop fields throughout a broad region from tropical to temperate zones (Walker et al. 1989; Panizzi and Rossi 1991). This weed is also naturalized in Africa, the Hawaiian Islands, India (Marjappan and Narayanaswamy 1972), Australia and the West Indies. It was introduced into Florida in ship ballast at Pensacola in the 1800s and is currently a problem in southern Alabama, southern Georgia, northern Florida, and isolated areas in central and south Florida. Bristly starbur is also reported as a weed in the Carolinas and Virginia and has been reported as far north as New Jersey.

Acanthospermum hispidum has many common names including bristly starbur, goat's-head, starbur, corona de la reina, sling shot weed and cuagrilla. The scientific name of the genus, *Acanthospermum*, is from the Greek words acantha (thorn) and sperma (seed) and refers to the fruit. *Hispidum* Latin and means rough, shaggy, prickly or bristly and refers to the numerous hairs covering the plant (Hall et al. 1991). Bristly starbur is an upright annual with dichotomous (Y-shaped) branching which gives the plant one of it's common names – slingshot weed. Cronquist (1980) has described bristly starbur as a summer annual with stems and leaves densely covered with stiff or soft hairs,

with erect stems, 1-3 feet tall, elliptic to ovate leaf blades, toothed or entire, mostly 2-10 cm long and 1-7 cm wide. The leaves are sessile and are opposite with glands on the abaxial leaf surface. Starbur flowers are typical of the Compositae (Asteraceae) Family, with heads 4-5 mm wide at anthesis (with about 8 disc flowers), cuneate burs and strongly compressed. The fruits are flattened and triangular in shape, strongly compressed and covered with stiff, hooked hairs. A straight or curved pair of spines (3-4 mm long) occur at the top of the fruit. Each fruit, excluding the terminal spines, is 5-6 mm long. The terminal spines are strongly divergent and approximately 4 mm long. Seed dispersion occurs via the hooked hairs, which can easily attach to the coats of animals. The bristly appearance and grouping of several fruits in each head provides the most frequently used common name, bristly starbur. The terminal spines combined with the triangular shape of the seed provide an additional common name, goathead. The plant is not considered useful due to the presence of toxics, which prevent it's use as forage and it has been shown to be toxic to goats and mice (Ali and Adam, 1978).

Seed production is prolific with higher seed production when seedlings emerged early in the cropping season. Seed production continues until the plant freezes in fall (Schwerzel 1970). Seed germination is variable and seeds appear to possess dormancy (Schwerzel, 1970). It is speculated that a combination of factors such as an immature embryo, impermeable seed coat, and substances inhibiting germination contribute to the dormancy of the seeds (Mayer et al. 1963). Studies also suggest that burial decreases viability and plowing the seed > 75 mm deep may help eliminate viable seed supply in the soil (Schwerzel, 1976).

This weed is a major problem in corn, peanuts and soybeans in Florida because it directly competes with moisture, light and nutrients (Hall et al. 1991). This weed also increases the cost of production by interfering in harvesting, since it continues to grow until a killing frost (Vester 1974). Several studies also suggest bristly starbur is quite competitive. In peanut, full season interference of bristly starbur from 8, 16, 32, and 64 plants per 7.5 feet of row reduced peanut yields by 14, 26, 43, and 50% respectively (Hauser et al. 1975). Chemical control is one of the most important methods for bristly starbur, as a weed-free period of 6 to 8 wk has been shown to optimize peanut yield (Schipper, 1997). Most peanut growers use a standard weed management program, which includes preplant incorporated (PPI) application of a dinitroaniline and/or a chloroacetamide herbicide to control grasses and small-seeded broadleaf weeds followed by a preemergence (PRE) or at-cracking (AC) application to control broadleaf weeds and escaped grasses. To control late-season bristly starbur, postemergence (POST) applications of chlorimuron are often required. In cotton, bristly starbur is managed by postemergence applications of MSMA, directed applications of diuron or prometryn plus MSMA, or more recently, glyphosate in Roundup Ready cotton varieties.

Fluazifop-p-butyl (heretofore referred to as fluazifop) is a post gramicide herbicide that selectively controls grass weeds in several broadleaf agronomic and horticultural crops (Haga et al. 1987). Fluazifop was first tested for herbicidal activity by ICI Americas in the U.S in 1981 (WSSA Herbicide Handbook, 2002). Selectivity is based on inhibition of the acetyl-CoA carboxylase (ACCase) enzyme in grasses, which is the initial step in fatty acid synthesis (Rendina and Felts 1988; Secor and Cseke 1988; Burton et al. 1989; Di Tomaso et al. 1993). In broadleaves and sedges, the ACCase enzyme is

not sensitive to fluazifop or other ACCase inhibiting herbicides. Konishi et al. (1996) reported broad-leaved plants have a prokaryote type ACCase, which is resistant to the herbicides in plastids. Grasses contain an eukaryote-type ACCase that is susceptible to these herbicides.

Fluazifop is the active ingredient in several herbicides which are registered for use at 0.05- 0.21 kg ai/ha for control of annual and perennial grasses. Grasses controlled include: barnyardgrass [*Echinochloa crus galli (L.*) Beauv.], Bermudagrass [*Cynodon dactylon (L.*) Pers.], crabgrass spp. (digitaria spp.), downy brome [*Bromus tectorum (L.*), *Panicum spp.*, foxtail spp. (*Setaria spp*.), volunteer cereals, shattercane [*Sorgum bicolor (L.*) Moench], quackgrass [*Agropyron repens (L*.) Beauv.], and Johnsongrass (*Sorghum halepense (L.*) Pers. #[3] SOHRA]. It is labeled for use in many broadleaf agronomic and horticultural crops such as cotton [*Gossypium hirsutum (L.)*], soybeans [*Glycine max (L.*) Merr.], stonefruits (*Prunus spp.*), asparagus (*Asparagus officinalis L*.), carrots (*Daucus carota L.*), garlic (*Allium sativum L*.), Coffee (*Coffea arabica L.*), endive (*Cichorium intybus L*.), pecans [*Carya illinoinensis (*Wangenh.) K Koch], rhubarb (*Rheum rhabarbarum L.*), and Tabasco peppers (*Capsicum frutescens L*.)(WSSA Herbicide Handbook, 2002).

Typical symptomology of fluazifop includes immediate cessation of growth after application, followed by leaf chlorosis within one to three weeks. Concurrently, the leaf sheaths become mushy, brown, and necrotic at the nodal attachment. Fluazifop diffuses readily across the plasmalemma and is rapidly deesterified to fluazifop acid. The acid then dissociates in the relatively alkaline cytoplasm. The anion's negative charge and low lipophilicity renders it immobile to traverse across plasmalemma. Thus there is "ion

trapping" and build-up of fluazifop in the symplasm (WSSA Herbicide Handbook, 2002). The acid principally translocates in the phloem and accumulates in meristematic regions of the root and shoot, where it disrupts the synthesis of lipids in susceptible species (Urano 1982; Erlingson 1988). Inhibition of fatty acid synthesis blocks the production of phospholipids used in building new membranes required for cell growth. Lipids are important components of cellular membranes and are produced in insufficient quantities causing a loss in membrane integrity. This occurs predominantly in meristematic regions and eventually the cell bursts or leaks and die. As a result, the growth ceases soon after application effecting young and actively growing tissues first, followed by leaf chlorosis with brown and necrotic tissues at the nodes 1-3 weeks after application (Rendina and Felts 1988; Secor and Cseke 1988).

Insensitive ACCase is not responsible for cross-resistance to a number of herbicides including fluazifop in rigid ryegrass *(Lolium rigidum*) from Australia (Powles et al, 1990). Rather it is believed that resistance may be due to increased herbicide metabolism or by sequestration away from the site of action (WSSA Herbicide Handbook 2002).

Fluazifop can be applied post emergence, which requires surfactant or crop oil for maximum efficacy. Fluazifop shows negligible losses due to volatilization and photodegradation in field conditions (WSSA Herbicide Handbook 2002). Fluazifop-P butyl ester is completely metabolized in susceptible plants to the phytotoxic fluazifop-p acid. Studies show that quackgrass retained 46-79% of applied fluazifop as the acid after 48 h, whereas a small fraction was metabolized to polar and nonpolar conjugates. The

acid takes longer to degrade in the susceptible plants, with residues remaining up to 45 days after treatment (Coupland 1985).

Gessa et al. (1987) found that fluazifop can bind irreversibly with certain clay soils by several different mechanisms. Kulshrestha et al. (1995) reported despite strong adsorption to soil particles, fluazifop was shown to leach to at least 15 cm deep in soybean fields in India. Conversely, fluazifop is reported to be of low mobility in soils and does not present an appreciable risk of groundwater contamination (WSSA Herbicide Handbook 2002). Fluazifop-p-butyl breaks down rapidly in moist soils with a half- life of generally less than one week. The major degradation product, fluazifop acid breaks down fairly rapidly with a half-life of three weeks.

Dicotyledonous plants are generally tolerant to aryloxyphenoxypropionate (AOPP) and cyclohexanedione (CHD) herbicides (Devine and Shimabukuro 1994), (Harwood 1991). However, a study by Luo and Matsumoto (2002) suggested that bristly starbur (*Acanthospermum hispidum DC.*), was found to be very susceptible to fluazifop. They also reported this species to be tolerant to other AOPP herbicides (quizalofop-ethyl and fenoxaprop-ethyl) and to the CHD herbicide, sethoxydim. Other compositae weeds including small flower galinsoga (*Galinsoga parviflora Cav.)*, annual sowthistle (*Sonchus oleraceus L.*), and hairy beggarticks (*Bidens pilosa L.*) were tolerant to fluazifop (Luo and Matsumoto). Interestingly, the period necessary for appearance of phytotoxic symptoms and seedling death in bristly starbur was much shorter than that in oat (*Avena sativa L.*), a susceptible grass species (Luo and Matsumoto 2002, Luo, Matsumoto and Usui 2001). Although fluazifop increased electrolyte leakage from shoots

of both species suggesting membrane disruption, greater leakage was observed from bristly starbur.

Similar observations were also made by other studies. County agents in Georgia observed control of bristly starbur during routine use of fluazifop in grower fields. Studies confirmed this activity of fluazifop on bristly starbur under greenhouse conditions and showed that the mode–of-action and symptomology associated with this activity was contact in nature, atypical of fluazifop (Teuton et al. 2002). Symptomology was much more rapid with desiccation and necrosis within 4-5 days after planting, suggesting an alternative mode-of-action than that associated with the regular activity in grasses. It was also confirmed that bristly starbur was only susceptible to fluazifop, but tolerant to other AOPP herbicides and CHD herbicides. There was also no difference between technical and commercial formulations, suggesting the active ingredient fluazifop was responsible for the activity.

Collectively, these studies suggested an alternative mechanism-of-action for fluazifop on bristly starbur, more specifically rapid membrane disruption. Study by Luo et al. (2002) showed that fluazifop-butyl caused membrane peroxidation in bristly starbur. Ethylene evolution and membrane lipid peroxidation in the plant seedlings were also investigated and results strongly suggest the primary mechanism was directly on membranes and active oxygen species and/or free radicals were involved in peroxidation. However, these studies failed to clarify that at what cellular level this membrane peroxidation might have been taking place. Therefore, studies were conducted on ion leakage and chlorophyll fluorescence to further elucidate the action mechanism of fluazifop in bristly starbur.

Glyphosate Tolerant Cotton and Trimesium Salt of Glyphosate

Poor weed control has been cited as one of the greatest limitation to successful cotton (Gossypium hirustum L.) production (McWhorter and Jordon 1985). Recent technological advancements in new post emergence over-the-top (POT) herbicide options have allowed cotton producers to explore total POT weed management (Culpepper and York 1997, 1999; Wilcut et al. 1996). Registered herbicides for POT weed broadleaf weed control in cotton include glyphosate in (Round-up Ready™ varieties) in (BXN ™ varieties), bromoxynil, MSMA, and pyrithiobac (Culpepper and York 1997, 1999; Jordon et al. 1997; Wilcut and Askew 1999).

Glyphosate herbicide has been primarily used in cropping systems as a burndown herbicide applied in minimum tillage operations before the introduction of glyphosate resistant crops. Glyphosate effectively controls many dicotyledonous and monocotyledonous weeds common to agronomic crops. Glyphosate is foliar active only and rapidly inactivated in the soil. Symptomology includes inhibited growth soon after application followed by general foliar chlorosis and necrosis within 7 to 21 days. Pronounced chlorosis may appear first in immature leaves and growing points. Once absorbed across the cuticle, glyphosate enters the phloem where it is distributed symplastically along with the usual contents of the sieve elements, with accumulation in underground tissues, immature leaves and meristems. (Gougler et al. 1981; Kleier et al. 1988; Lichtner et al. 1984; Neuman et al. 1985; Tyree et al. 1979). Apoplastic translocation has been observed in quackgrass, but most research suggest little to no apoplastic movement (WSSA Herbicide Handbook 2002). Glyhosate acts by inhibits 5-enolpyruvylshikimate 3-phosphate (EPSP) synthase (Amrhein et al. 1980) in the shikimic acid pathway, which leads to depletion of the aromatic amino acids tryptophan, tyrosine,

and phenylalanine. This leads to the arrest of protein production and prevention of secondary product formation (Franz, Mao and Sikorski 1997). The deregulation of the shikimate pathway also leads to general metabolic disruption (Duke et al 2003) (1988).

Cotton (*Gossypium hirustum L.*) varieties resistant to glyphosate were introduced in 1997 to growers in United States (Nida et al. 1996, Heering et al. 1998). Round-up Ready$^{(TM)1}$ cotton is resistant to vegetative injury from glyphosate herbicide, but detrimental effects on reproductive development may occur if glyphosate is applied beyond the four-leaf stage. Since commercialization in 1997, concerns have been raised about the reproductive tolerance of glyphosate resistant cotton to glyphosate. Numerous reports of increased boll abscission in response to glyphosate treatments have been reported (Jones and Snipes 1999; Vargas et al. 1998).

Effective weed management is very critical to maximize cotton yields and retain high quality harvest. Cotton is highly sensitive to early season weed competition (Culpepper et al. 1998; Scott et al. 2001). In addition to competition, various weeds may act as hosts for insect pests which can infest cotton. Glyphosate-resistant cotton allows growers broader spectrum of weed control as compared to other herbicide systems, as well as adds greater flexibility in herbicide applications (Askew and Wilcut 1999; Culpepper and York 1999; Scott et al.2001). It also makes this crop very cost effective as compared to conventional cotton, as it reduces the and other intensive operations in cotton production.

Several crops resistant to glyphosate have been commercialized since the mid-1990s' (Duke, Scheffler, Dayan and Dyer 2002, Thayer 2000). Glyphosate resistance is

[1] Monsanto Co., St.Louis, MO 63167

conferred to cotton by the incorporation of a glyphosate-insensitive EPSP synthase [EC 2.5.1.19] gene cloned from Agrobacterium spp. strain CP4 (CP4-EPSPS). The expression of the CP4-EPSPS gene produces a GR EPSPS enzyme, which can overcome the inhibitory effects of native EPSPS in the presence of glyphosate. This allows sufficient production of aromatic amino acids and secondary metabolites in shikimic acid pathway (Nida et al. 1996).

Glyphosate typically accumulates in tissues that act as metabolic sinks (Gougler and Geiger 1981; Sandberg et al. 1980). Glyphosate may cause damage if accumulation in reproductive tissues exceeds beyond the tolerance threshold. This is because rapidly developing reproductive tissues have increased demands of carbohydrates and amino acids, in addition to sensitivity to biotic and abiotic factors. Therefore the timing of glyphosate treatments to glyphosate tolerant cotton is very critical, relative to the development of reproductive organs (Pline et al. 2001).

Glyphosate (Roundup Ultra) is formulated as an isopropylamine salt (IPA) of N-phosphonomethyl glycine, and this formulation was the only one initially registered for use on Round-up-Ready™ soybeans and cotton. The trimethylsulfonium salt formulation of glyphosate was approved for commercial use on glyphosate-resistant soybean in 1999. Environmental Protection Agency (EPA) registered Touchdown[2] herbicide formulated as a potassium salt in 2000 for use over Round-up-Ready™ soybeans, cotton and corn as a replacement for the trimethylsulfonium salt formulation (Wallaces Farmer 2002). This new formulation does not cause injury unlike the trimethylsulfonium salt formulation on Round-up-Ready™ cotton. Injury was observed with the (TMS)

[2] Syngenta Crop Protection Inc., Greensboro, NC- 27409.

trimethylsulfonium salt formulation used in original Touchdown formulation and was never labeled for Round-up-Ready™ cotton. The TMS salt has been subsequently identified as the cause of injury in RR cotton (McGraw et al, 2001).

The trimethylsulfonium (TMS) salt of glyphosate has become an alternative to the IPA salt of glyphosate since the early 1990's. Similar toxicities and weed control have been reported with both formulations. There were no differences between formulations because both formulations were ionized to the same active, glyphosate acid. However, trimethylsulfonium salts may affect plant growth and research has demonstrated that phytotoxic effects have been observed on several plant species. The difference in duckweed (Lemna gibba L.) control between the TMS and IPA formulations of glyphosate was caused by the TMS portion of the formulation. (Sùrensen and Gregersen 1999) also suggested that the lethal mechanism between glyphosate and trimethylsulfonium salts of glyphosate may be different.

Previous observations indicated moderate to severe injury when the TMS formulation of glyphosate was applied to Round-up Ready™ cotton. The effects of glyphosate formulation on Round-up-Ready™ cotton were studied under field conditions and also showed severe injury with the TMS formulation. However dissimilar symptomology to glyphosate was observed on non-transgenic and glyphosate tolerant cotton. The TMS formulation caused leaf chlorosis followed by necrosis and severe stunting within 5 to 7 days, while normal symptoms of glyphosate injury are not generally visible until 10 days after application. The TMS formulation of glyphosate also caused over an 80% reduction in the photosynthetic rates of both conventional and Round-up-Ready™ cotton (MacDonald et al. 2001). The IPA formulation of glyphosate

also reduced photosynthetic rates in conventional cotton but did not affect the Round-up-Ready™ variety.

Further studies under greenhouse conditions investigated the effect of trimethylsulfonium iodide, (TMS) without glyphosate and several formulations of glyphosate including the isopropylamine salt, TMS, sesquesodium salt, ammonium salt and non-formulated technical acid applied to cotton at the 4th leaf stage. The TMS glyphosate formulation, the trimesium iodide alone, and in combination with technical glyphosate acid caused a significant reduction in the photosynthetic rates of Round-up-Ready™ cotton (MacDonald et al 2001). The symptomology observed was similar to that observed under field conditions. These studies indicated that there was an alternate mode of action of TMS in Round-up-Ready™ cotton and suggest that the trimethylsulfonium salt itself was phytotoxic to cotton. The symptomology and rapid reduction in photosynthesis rates further suggested that the salt may be a photosynthetically active compound. Therefore, studies were conducted on ion leakage and chlorophyll florescence to further characterize the activity of trimethylsulfonium salt of glyphosate on Round-up-Ready™ cotton.

CHAPTER 2
FLUAZIFOP-BUTYL ACTIVITY ON BRISTLY STARBUR

Introduction

Bristly Starbur (*Acanthospermum hispidum DC.*) is an annual, non-native weed from central and South America (Vester 1974). Bristly starbur is found throughout Central America to southward Argentina in South America (Hall et al. 1991). It is categorized as one of the main weeds in many crop fields throughout a broad region from tropical to temperate zones (Walker et al. 1989; Panizzi and Rossi 1991). This weed is also naturalized in Africa, the Hawaiian Islands, India (Marjappan and Narayanaswamy 1972), Australia and the West Indies. It was introduced into Florida in ship ballast at Pensacola in the 1800s and is currently a problem in southern Alabama, southern Georgia, northern Florida, and isolated areas in central and south Florida. Bristly starbur is also reported as a weed in the Carolinas and Virginia and has been reported as far north as New Jersey.

Bristly starbur is an upright annual with distinctive dichotomous (Y-shaped) branching, reaching 1-3 feet in height. Cronquist (1980) has described bristly starbur as a summer annual with stems and leaves densely pubescent. The fruits are flattened and triangular in shape and the bristly appearance and grouping of several fruits in each head provides the most frequently used common name, bristly starbur. The plant is not considered useful due to the presence of toxins and has been found to be toxic to goats and mice (Ali and Adam, 1978).

Bristly starbur is a major problem in corn, peanuts and soybeans throughout the southeastern U.S, because it directly competes for moisture, light and nutrients in the soil (Hall et al. 1991). This weed also increases the cost of production by interfering in harvesting and contaminated lint (Vester 1974). Several studies also suggest bristly starbur is quite competitive. In peanut, full season interference of bristly starbur from 8, 16, 32, and 64 plants per 7.5 feet of row reduced peanut yields by 14, 26, 43, and 50% respectively (Hauser et al. 1975).

Fluazifop-p-butyl is a post gramicide herbicide that selectively controls grassy weeds in several broadleaf agronomic and horticultural crops (Haga et al. 1987). It is registered for use under several trade names for the control of both annual and perennial grasses. Grasses controlled include: barnyardgrass [*Echinochloa crus galli (L.*) Beauv.], Bermudagrass [*Cynodon dactylon (L.*) Pers.], crabgrass spp. (*digitaria spp.*), downy brome [*Bromus tectorum (L.*), *Panicum spp.,* foxtail spp. (*Setaria spp.*), volunteer cereals, shattercane [*Sorgum bicolor (L.)* Moench], quackgrass [*Agropyron repens (L.*) Beauv.], and Johnsongrass (*Sorghum halepense (L.*) Pers. #[3] SOHRA].

Typical symptomology of fluazifop includes immediate cessation of growth after application, followed by leaf chlorosis within one to three weeks. Concurrently, the leaf sheaths become mushy, brown, and necrotic at the nodal attachment. Fluazifop-p-butyl diffuses readily across the plasmalemma and is rapidly deesterified to fluazifop acid which dissociates in the relatively alkaline cytoplasm. The anion's negative charge and low lipophilicity renders it immobile to traverse across plasmalemma, and build-up of fluazifop occurs in the symplasm (WSSA Herbicide Handbook, 2002). The acid principally translocates in the phloem and accumulates in meristematic regions of the root

and shoot, where it disrupts the synthesis of lipids in susceptible species (Urano 1982; Erlingson 1988). Specifically, fluazifop inhibits the enzyme acetyl Co-A carboxylase which leads to an inhibition of fatty acid synthesis. This blocks the production of phospholipids used in building new membranes required for cell growth. Lipids are produced in insufficient quantities causing a failure of membrane integrity and eventually the cell leaks and dies (Rendina and Felts 1988; Secor and Cseke 1988). An insensitive ACCase provides the tolerance mechanism for broadleaf plants. However, insensitive ACCase is not responsible for the development of resistance to a number of herbicides including fluazifop in rigid ryegrass (Lolium rigidum) from Australia (Powles et al, 1990).

Dicotyledonous plants are generally tolerant to aryloxyphenoxypropionate (AOPP) and cyclohexanedione (CHD) herbicides (Devine and Shimabukuro 1994), (Harwood 1991). However, a study by Luo and Matsumoto (2002) suggested that bristly starbur (Acanthospermum hispidum DC.), was found to be very susceptible to fluazifop. They also reported this species to be tolerant to other AOPP herbicides (quizalofop-ethyl and fenoxaprop-ethyl) and to the CHD herbicide, sethoxydim. Other Compositae weeds including: small flower galinsoga (*Galinsoga parviflora*), annual sowthistle (*Sonchus oleraceus*), and hairy beggarticks (*Bidens pilosa*) were shown to be tolerant to fluazifop (Luo and Matsumoto). Interestingly, the period necessary for appearance of phytotoxic symptoms and seedling death was much shorter than in oat (*Avena sativa L.*), a susceptible grass species (Luo and Matsumoto 2002) (Luo, Matsumoto and Usui 2001). Although fluazifop increased electrolyte leakage from shoots of both species suggesting membrane disruption, greater leakage was observed from bristly starbur.

Similar observations were also made by county agents in Georgia, who observed control of bristly starbur during routine use of fluazifop in grower fields. Studies confirmed this activity of fluazifop on bristly starbur under greenhouse conditions and showed that the mode–of-action and symptomology associated with this activity was contact in nature, atypical of fluazifop (Teuton et al, 2002). Symptomology was much more rapid with desiccation and necrosis within 4-5 days after treatment, suggesting an alternative mode-of-action than that associated with the regular activity in grasses. It was also confirmed that bristly starbur was only susceptible to fluazifop, but tolerant to other AOPP herbicides and CHD herbicides. There was also no difference between technical material and the commercial formulation, suggesting the active ingredient fluazifop was responsible for the activity.

Collectively, these studies suggested an alternative mechanism-of-action for fluazifop on bristly starbur, more specifically rapid membrane disruption. A study by Xiao Yong Luo et al. (2002) showed that fluazifop-butyl caused membrane peroxidation in bristly starbur. Ethylene evolution and membrane lipid peroxidation on plant seedlings were also investigated and results strongly suggest the primary mechanism was directly on membranes and active oxygen species and /or free radicals were involved in peroxidation. However, these studies failed to clarify at what cellular level this membrane peroxidation might be taking place. Therefore, studies were conducted on ion leakage and chlorophyll fluorescence to further elucidate the mechanism of action of fluazifop in bristly starbur.

Material and Methods

General Procedures

Bristly starbur seeds were collected from naturally occurring populations in Gainesville, Florida. Unless otherwise stated, the plants were grown as follows. Seeds were wrapped in muslin cloth and placed under continuous running water for 6-7 days. Seeds were planted in potting soil (Metro-mix 200)[1] and placed under greenhouse conditions at Gainesville, Florida. The plants reached the 3-4 leaf stage after 2-3 weeks, at which time leaves were harvested for experimental use. Plant material for all experiments consisted of 0.7 cm^2 leaf discs. After cutting leaf discs were placed in distilled water for approximately one hour to allow for callus formation.

Chemicals

Technical grade fluazifop (butyl 2-(4-((5-(trifluoromethyl)-2-pyridinyl) oxy) phenoxy) propanoate)) was obtained from Chem Service Inc.[2]. Gramicidin, 2, 4 DNP (2, 4 dinitrophenol), paraquat (1, 1'-dimethyl-44'-bipyridinium dichloride) and diuron (3-(3, 4-dichlorophenyl)-11-dimethylurea) were obtained from Sigma Chemicals Co[3]. Due to low water solubility, fluazifop, gramicidin and diuron were dissolved initially in small volume of ethanol and then diluted to required concentrations with distilled water. Final ethanol concentrations were 1-2 % (v/v) and did not affect leaf tissues. 2, 4 DNP and paraquat concentrations were made directly by dissolving in distilled water. Unless otherwise noted, an experimental unit consisted of one 20 ml scintillation vial containing 8 leaf discs and 10 ml of treatment solution.

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[1] Scotts Agricultural. Products, Marysville, OH 43041

[2] Chem Service Inc. West Chester, PA 19381-0599

[3] Sigma Chemicals Co., West Chester, PA 19381

Rate Studies

To calculate an I_{50} concentration of fluazifop on bristly starbur, rate studies were performed under both light and dark conditions. Ion leakage was measured as percent conductivity (µmhos) over time as a function of concentration. For light experiments, vials were placed under continuous light (80- 120 $\mu mol.m^{-2}.s^{-1}$) at 21 C in a water bath. Concentrations tested were 10, 100, 250, 500, 750, and 1000 µM with a distilled water control. Conductivity was measured initially and 12, 24, 36, 48, 72, 96, 120, 144, and 168 hours after initial exposure. For dark experiments, vials were placed on a shaker bath in the dark at 25 C. Treatment parameters were identical to light studies except conductivity measurements were stopped at 120 hours.

Total conductivity was obtained by freezing and thawing the solutions twice to release all ions. Data are presented as percent conductivity derived from the following equation:

% conductivity = ((measured – initial)/ (total – initial))* 100

where measured equaled the amount of conductivity at the time of measurement.

Comparison Studies

The activity of fluazifop was compared to compounds with known modes of action to gain better understanding of the mechanism of fluazifop on starbur. Treatments consisted of fluazifop at 600 µM (based on $I_{50.}$ calculations from rate studies), diuron (100 µM), 2, 4 DNP (50 µM), gramicidin (10 µM) and paraquat (10 µM). These studies were conducted under both light and dark conditions for a total of 120 hours. Experimental conditions were the same as previously described for the rate study.

Fluorescence

Twenty leaf discs were placed in 25ml erlenmeyer flasks containing 10 ml of treatment solution and vacuum infiltrated. Treatments consisted of fluazifop at 600 μM (based on $I_{50.}$ calculations from rate studies), diuron (100 μM), 2,4 DNP (50 μM), gramicidin (10 μM) and paraquat (10 μM). Leaf discs were placed under continuous light (120 μmol.m-2.s-1) at 21 C in a water bath. Fluorescence[4] was measured at 30, 60, 90, 150, and 210 minutes. Treated leaf discs were dark-equilibrated for 10 minutes prior to chlorophyll 'a' fluorescence determination and initial, peak and terminal fluorescence measurements were taken. Terminal fluorescence was measured after 50 seconds with a 1.0 second gain between initial and peak measurements. Data are presented as the ratio of peak to terminal fluorescence derived from the following equation:

Peak / terminal ratio = (peak – initial)/ (terminal – initial)

Results are means of six replications.

Statistics

Unless otherwise noted, all treatments contained a minimum of 4 replications and studies were conducted twice. Data was subjected to analysis of variance (ANOVA) to test for interactions and treatment effects ($P < 0.05$). Results for the studies are presented with standard errors of the mean. There was no significant interaction between experiments for all studies, therefore data was pooled.

[4] Plant Productivity Fluorometer, Model SF20, Richard Brancker Research, Ottawa, Ontario, Canada.

Results and Discussion

Rate Studies

At concentrations < 250μM, fluazifop caused less than 30 % leakage after 96 hours of exposure. Fluazifop caused slightly higher leakage at 120 hours and > 40 % at 144 hours.A significant decrease in conductivity was measured at 168 hours, this was thought to be caused by complexing of some ions. Overall there was minimal difference between rates and 500, 750 and 1000 μM after 120 to 144 hours in light conditions, and > 50 % leakage was observed at 144 hours. Under dark conditions there was significant leakage with all concentrations after 24 hours, with concentrations ≥ 250 μM causing more than 60% leakage after 24 hours (Figure 2-2). The rate which caused 50 % ion leakage was significantly lower in dark compared to light (Table 2-2). There was a decreasing effect of rate as exposure time increased, however > 90 % ion leakage was observed for all concentrations after 96 hours of exposure time in dark. A calculated I 50 value at 96 to 120 hours was 600 μM based on the data from light studies.

Comparison Studies

Fluazifop caused more than 60% ion leakage after 96 hours under light conditions, which was similar to that observed from diuron (Figure 2-3). 2, 4 DNP and gramicidin caused a minimal increase in conductivity (< 20 %) under light conditions, with only a slight increase in conductivity over the treatment period (Table 2-3).

Paraquat caused the most rapid increase in conductivity in the light, producing over 70 % total leakage from bristly starbur leaf discs after 12 hours of initial exposure. Percent conductivity caused by fluazifop, paraquat and diuron increased over time with paraquat causing nearly 95% conductivity after 96 hours under continuous light. The ion

leakage of diuron and fluazifop was similar after 72 hours (approximately 65 % conductivity), although the leakage from fluazifop was slightly greater than diuron.

The effect of fluazifop was again much more rapid under dark conditions compared to those observed in the light regime and resulted in higher percent conductivity values (nearly 75%) after 72 hours of treatment (Figure 2-4). Gramicidin caused similar leakage under dark conditions with less than 10% conductivity, which slightly increased over the treatment period. The leakage caused by 2, 4 DNP in dark was slightly higher, as compared to that in light (Table 2-4). Paraquat caused less conductivity under dark conditions with nearly 50% conductivity observed after 48 hours. Diuron also caused less ion leakage under dark conditions, causing (<20 %) leakage over time.

2, 4 DNP and paraquat are known to affect both photosynthesis and respiration, injuring the cells through two independent mechanisms. 2, 4 DNP is an uncoupler of respiration, whereas in the dark only respiration is effected. Leakage caused by paraquat was much greater in light compared to dark primarily due to the formation of membrane disrupting radical oxygen species. Although disruption of respiration did probably occur, the impact of paraquat is much greater in the light. The opposite occurred from 2, 4-DNP where greater leakage was observed in the dark, especially after 144 hours. This indicates 2, 4-DNP is a more potent respiratory inhibitor than photosynthetic inhibitor. Although some oxidative stress is induced by inhibition or diversion of oxidative electron flow, the major cause of membrane disruption is the collapse of the membrane gradient due to lack of energy.

Diuron is a photosynthetic inhibitor that acts by blocking electron transport; more specifically by binding to the Q_B-binding niche on the D1 protein of photosystem II. This

stops the production of ATP and $NADPH_2$. The inability to transfer energy promotes the formation of triplet chlorophyll and singlet oxygen, which induces lipid peroxidation and in leaky membranes. This causes cells to disintegrate rapidly. This compound has no effect on respiration and this is reflected in the low percent conductivity values in dark. Fluazifop appears to be acting similarly to 2, 4-DNP and gramicidin in the dark; the increase in the activity in the dark conditions correlates with compounds that only inhibits respiration. The effect is less under light conditions because photosynthesis would provide some energy for respiration, thus diminishing activity. Gramicidin directly affects respiration but has little influence on photosynthesis. This compound also caused greater ion leakage in the dark.

Fluorescence

Fluazifop, diuron and paraquat decreased the peak / terminal ratio, relative to distilled water control (Figure 2-5) indicating an increase in chlorophyll 'a' fluorescence at 0.5, 1, 1.5, 2.5 and 3.5 hours after treatment. 2, 4 DNP and gramicidin did not affect fluorescence until one hour after treatment, probably due to indirect effects caused by membrane disruption (Table 2-5).

Chlorophyll 'a' fluorescence is a direct measure of light reaction efficiency where chlorophyll molecules re-radiate 'excess' absorbed light energy as fluorescence (Lawlor 1987). Chlorophyll fluorescence is usually measured through the ratio of peak to terminal fluorescence. In normal light reactions, light is absorbed by chlorophyll and other pigments and transmitted to reaction centers where light energy is converted to chemical energy through the donation of electrons.

However, not all of the energy absorbed by chlorophyll molecules can be utilized and some is re-radiated as fluorescence. Normal fluorescence values for the ratio of peak to terminal for my study ranged from 8-12. This ratio indicates the ability of the plant to utilize light energy with higher ratios corresponding to more efficient light use, while low ratios (near 1.00) indicate that most of the energy is being lost to fluorescence.

Paraquat also produced low ratios, but this was probably due to the degradation of the photosynthetic apparatus by oxygen radicals.

Diuron produced characteristically low ratios over time, which is characteristic of compounds that block electron flow in photosystem II. Chlorophyll molecules continue to absorb light energy, and must re-radiate most of this energy as fluorescence to avoid photo-oxidation (Fuerst and Norman, 1991). The plants treated with these herbicides produce quantities of membrane-damaging triplet chlorophyll, singlet oxygen ($^1O_2$), superoxide (O_2^-), and hydrogen peroxide (H_2O_2).These radicals cause lipid peroxidation and subsequently destroy membrane integrity (Fuerst and Norman, 1991).

Gramicidin, an ionophore, which causes proton leakage from both chloroplast and mitochondrial membranes, also lowered peak/terminal ratio in conjunction with significant ion leakage. Gramicidin directly effects respiration but has little influence on photosynthesis. Therefore the effect observed on fluorescence is probably an indirect effect due to increased ion leakage and the disruption of photosynthetic apparatus. 2, 4 DNP behaved similarly to gramicidin. Fluazifop drastically decreased the peak / terminal ratio, which could be an indirect effect due to membrane disruption due to oxidative stress, as seen in the case of paraquat and 2, 4-DNP. Collectively this data suggests that fluazifop elicits rapid, membrane affects in bristly starbur. Greater and more rapid ion

leakage in the dark suggests that the activity of fluazifop on bristly starbur is due to respiratory inhibition.

Table 2-1 Ion leakage expressed as (%) conductivity when exposed to different concentrations of fluazifop over time (hours) in light.

	Time(hours)								
Treatments	12	24	36	48	72	96	120	144	168
0 µM	6.4[1]	8.5	9.8	10.5	11.5	10.8	12.5	18.5	18.0
10 µM	6.6	11.5	15.3	18.5	24.4	28.0	33.5	40.5	20.2
100 µM	6.9	11.5	10.5	13.0	18.5	23.0	31.0	45.6	17.2
250 µM	8.2	13.5	16.5	20.5	25.0	28.5	32.0	42.5	14.5
500 µM	9.2	16.5	20.0	25.5	32.5	36.0	39.8	65.0	38.5
750 µM	10.5	19.2	23.5	28.5	35.5	40.0	43.6	58.4	30.8
1000 µM	11.2	21.0	24.5	29.5	35.7	38.3	45.6	60.5	28.0

[1]Means of 8 replications.

Table 2-2. Ion leakage expressed as (%) conductivity when exposed to different concentrations of fluazifop over time (hours) in dark.

	Time (hours)						
Treatments	12	24	36	48	72	96	120
0 µM	2.7[1]	4.9	7.1	9.3	13.7	18.6	19.0
10 µM	21.2	55.5	70.8	84.0	90.9	96.1	90.0
100 µM	23.3	50.1	65.6	78.1	85.9	93.3	84.4
250 µM	22.9	61.6	71.4	80.3	87.8	94.1	85.1
500 µM	22.8	65.7	73.4	79.2	88.4	93.9	85.8
750 µM	36.9	70.2	77.8	84.9	93.8	98.9	88.4
1000 µM	50.8	73.7	76.6	84.5	93.7	98.8	88.7

[1]Means of 8 replications.

Table 2-3. The effect of diuron, 2, 4 DNP, gramicidin, paraquat and fluazifop on ion leakage in light.

	Time (hours)							
Treatments	12	24	36	48	72	96	120	144
Water (0 µM)	6.9[1]	9.0	10.4	12.6	17.2	16.4	14.4	10.5
Diuron (100 µM)	6.7	9.1	11.9	22.9	54.3	61.0	83.3	93.5
2,4 DNP (50 µM)	8.8	12.1	13.8	17.0	25.3	24.5	18.7	19.0
Gramicidin(10 µM)	7.4	9.9	10.7	13.8	23.0	22.3	19.9	14.4
Paraquat (10 µM)	71.0	81.3	81.5	89.0	90.8	94.3	96.3	97.2
Fluazifop(600 µm)	16.0	24.4	26.4	37.2	53.1	61.5	76.0	95.8

[1]Means of 8 replications.

Table 2-4. The effect of diuron, 2, 4 DNP, gramicidin, paraquat and fluazifop on ion leakage in dark.

	Time(hours)								
Treatments	12	24	36	48	72	96	120	144	168
Water (0 µM)	3.2[1]	4.0	4.7	5.7	7.7	8.9	14.7	22.4	46.5
Diuron (100 µM)	5.2	6.2	6.4	7.0	8.9	10.2	12.2	18.5	30.5
2,4 DNP (50 µM)	4.9	7.1	9.0	9.9	14.7	21.7	34.0	45.2	76.3
Gramicidin(10 µM)	3.4	4.5	5.0	5.4	7.2	10.1	18.5	27.0	52.5
Paraquat (10 µM)	5.6	22.7	43.0	46.4	54.4	77.5	85.9	86.5	80.1
Fluazifop(600 µm)	53.1	61.9	63.3	68.0	73.0	76.5	81.5	80.3	69.9

[1]Means of 8 replications.

Table 2-5. The effect of diuron, 2, 4 DNP, gramicidin, paraquat and fluazifop on chlorophyll 'a' fluorescence in bristly starbur leaf discs.

	Time(hours)				
Treatments	0.5	1.0	1.5	2.5	3.5
Water (0 µM)	7.9[1]	17.6	5.7	7.3	5.9
Diuron (100 µM)	2.4	2.9	2.1	1.2	1.7
2,4 DNP (50 µM)	10.5	4.6	7.6	7.7	5.1
Gramicidin(10 µM)	3.2	7.5	19.3	7.1	9.0
Paraquat (10 µM)	2.2	2.3	2.1	1.4	3.3
Fluazifop (600 µm)	1.1	1.1	1.1	1.4	2.0

[1] Peak/ Terminal Ratio

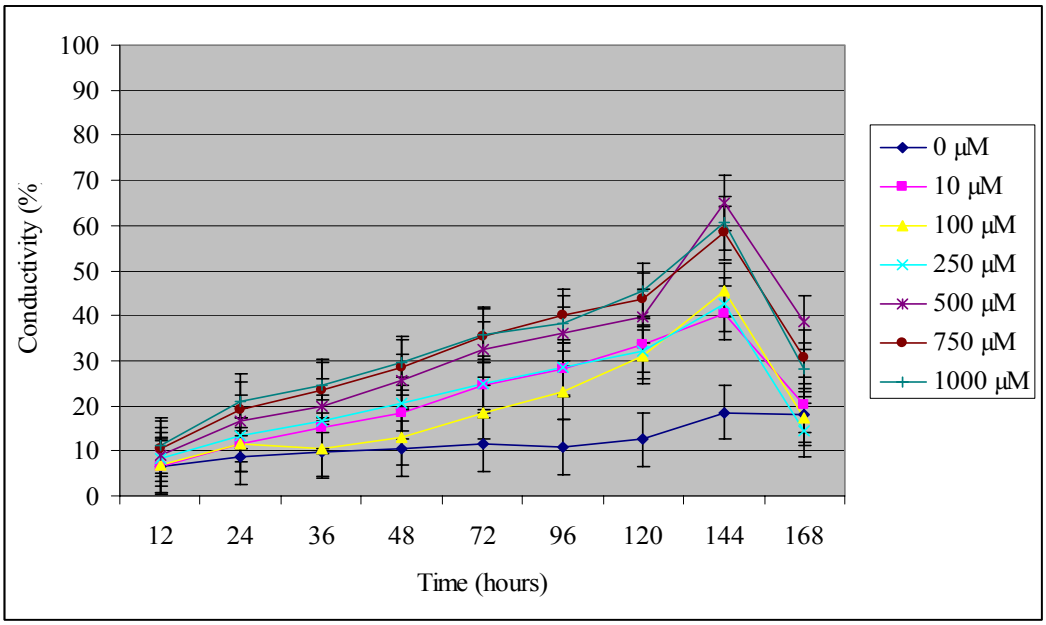

Figure 2-1. Ion leakage expressed as (%) conductivity when exposed to different concentrations of fluazifop over time (hours) in light.

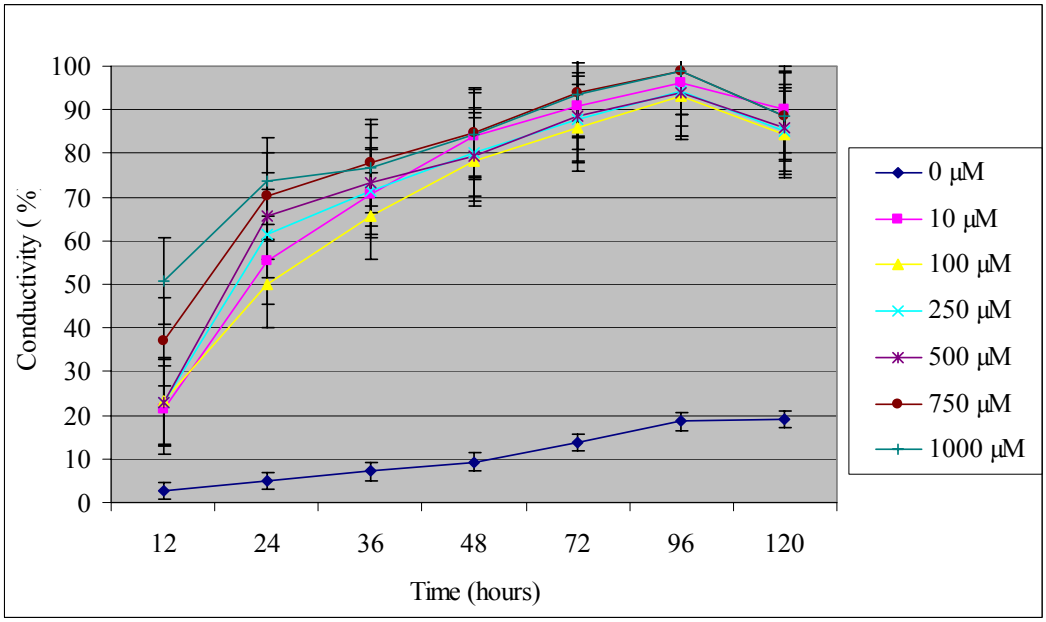

Figure 2-2. Ion leakage expressed as (%) conductivity when exposed to different concentrations of fluazifop over time (hours) in dark.

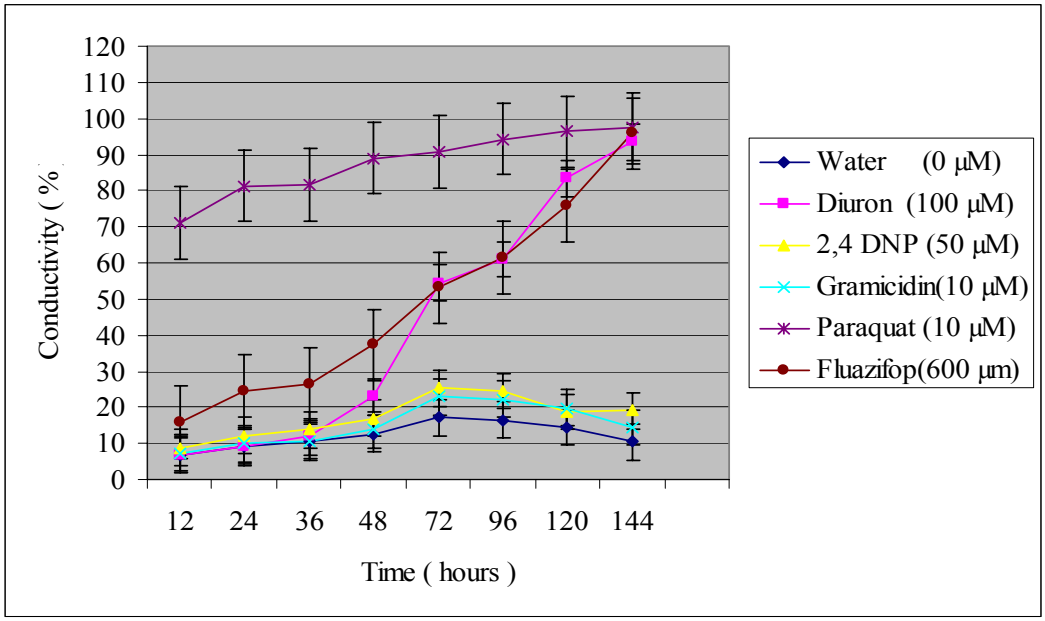

Figure 2-3. The effect of diuron, 2, 4 DNP, gramicidin, paraquat and fluazifop on ion leakage over time in light.

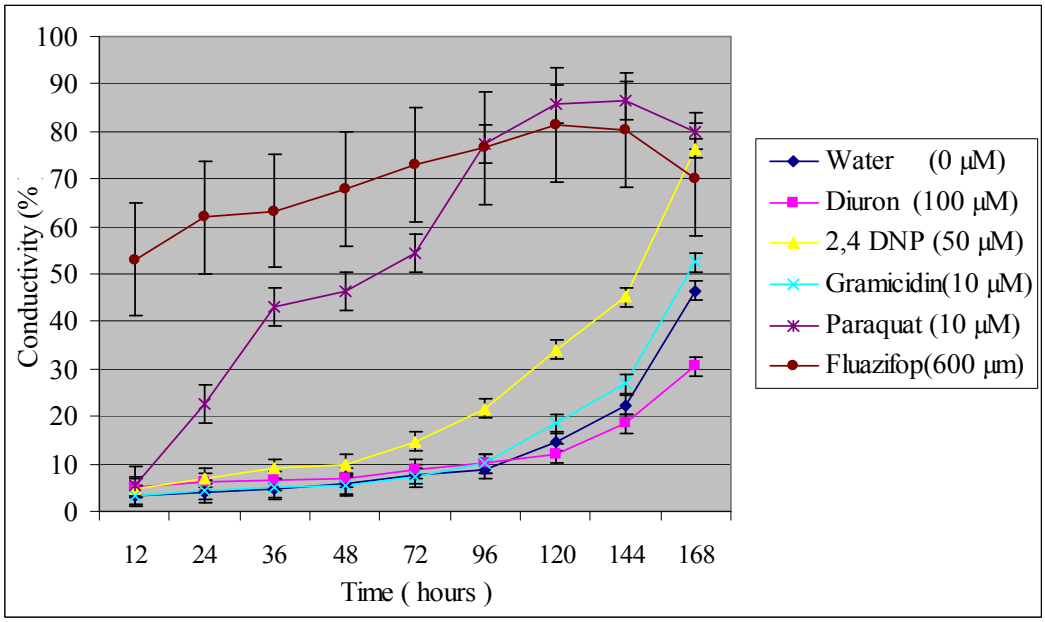

Figure 2-4. The effect of diuron, 2, 4 DNP, gramicidin, paraquat and fluazifop on ion leakage over time in dark.

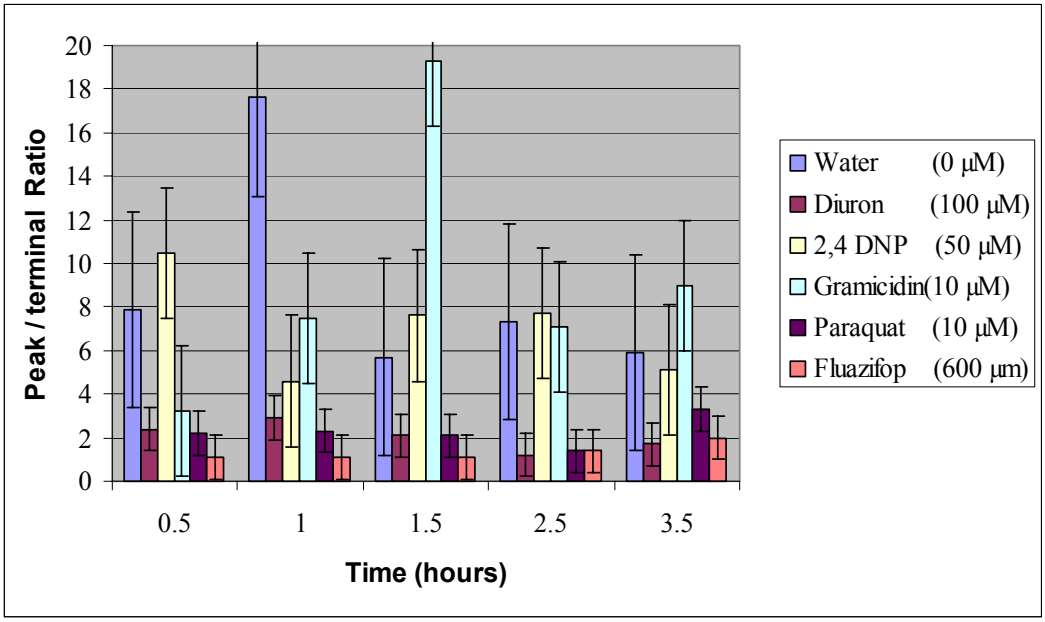

Figure 2-5. The effect of diuron, 2, 4 DNP, gramicidin, paraquat and fluazifop on chlorophyll 'a' fluorescence in bristly starbur leaf discs.

CHAPTER 3
TRIMETHYLSULFONIUM SALT FORMULATION OF GLYPHOSATE ACTIVITY ON ROUND-UP READY™ COTTON

Introduction

Glyphosate herbicide has been used since the 1970's in cropping systems as a burndown herbicide applied prior to planting in minimum tillage operations. Glyphosate effectively controls many dicotyledonous and monocotyledonous weeds common to agronomic crops. Glyphosate is foliar active only and rapidly inactivated in the soil. Symptomology includes inhibited growth soon after application followed by general foliar chlorosis and necrosis within 7-21 days. Pronounced chlorosis may appear first in immature leaves and growing points. Once absorbed across the cuticle, glyphosate enters the phloem and is distributed symplastically with accumulation in underground tissues, immature leaves and meristems (Gougler et al. 1981; Kleier et al. 1988; Lichtner et al. 1984; Neuman et al. 1985; Tyree et al. 1979). Apoplastic translocation is also seen in some plants like quackgrass, but most research suggest little to no apoplastic movement (WSSA Herbicide Handbook 2002). Glyhosate inhibits the enzyme 5-enolpyruvylshikimate 3-phosphate (EPSP) synthase (Amrhein et al. 1980) in the shikimic acid pathway, which leads to the depletion of aromatic amino acids subsequent protein production and secondary product formation is halted(Franz, Mao and Sikorski 1997) and deregulation of the shikimate pathway also leads to general metabolic disruption (Duke et al 2003, 1988).

Cotton *(Gossypium hirustum L.*) varieties resistant to glyphosate (Round-up Ready®)[1] were introduced in 1997 to growers in United States (Nida et al. 1996) (Heering et al. 1998). Round-up Ready™ cotton is resistant to vegetative injury from glyphosate herbicide, but detrimental effects on reproductive development may occur if glyphosate is applied beyond the four-leaf stage (Jones and Snipes 1999; Vargas et al. 1998). Since commercialization in 1997, Round-up ready varieties comprise > 80% of the cotton grown in the southeast U.S.

Cotton is highly sensitive to early season weed competition (Culpepper et al. 1998; Scott et al. 2001) and glyphosate-resistant cotton allows growers a broader spectrum of weed control as compared to other herbicide systems and adds greater flexibility in herbicide applications (Askew and Wilcut 1999; Culpepper and York 1999; Scott et al.2001).

Glyphosate (Roundup Ultra) is the isopropylamine salt (IPA) of N-phosphonomethyl glycine, and this formulation was the only one originally registered for use on Round-up-ReadyTM soybeans and cotton. The trimethylsulfonium salt formulation of glyphosate was approved for commercial use on glyphosate-resistant soybean in 1999, but never labeled on cotton due to excessive injury, which was attributed to the trimethylsulfonium salt (TMS) formulation used in original formulation. The trimethylsulfonium (TMS) salt of glyphosate has become a popular alternative to the IPA salt of glyphosate since the early 1990's. Similar toxicities and weed control have been reported with both formulations. There were no differences between formulations because both formulations were ionized to the same active glyphosate acid. However,

[1] Round-up Ready® Granular herbicide by Monsanto Co., St.Louis, MO-63167

trimethylsulfonium salts may affect plant growth and research has demonstrated that phytotoxic effects have been observed on several plant species. The difference in inflated duckweed (*Lemna gibba L.*) control between the TMS and IPA formulation of glyphosate was caused by the TMS portion of the formulation. Sùrensen and Gregersen (1999) suggested that the lethal mechanism between glyphosate and trimethylsulfonium salts of glyphosate may be different.

Previous research at the University of Florida has also confirmed moderate to severe injury when the TMS formulation of glyphosate was applied to Round-up Ready™ cotton, but with dissimilar symptomology to glyphosate injury. The TMS formulation caused leaf chlorosis followed by necrosis and severe stunting within 5 to 7 days, while normal symptoms of glyphosate injury are not generally visible until 10 days after application. The TMS formulation of glyphosate caused over an 80% reduction in the photosynthetic rates of both conventional and Round-up-Ready™ cotton (McGraw et al 2001). The IPA formulation of glyphosate also reduced photosynthetic rates in conventional cotton but did not affect the Round-up-Ready™ variety.

Further studies under greenhouse conditions investigated the effect of several formulations of glyphosate including isopropylamine salt, trimesium salt, sesquesodium salt, ammonium salt and non-formulated technical acid applied to cotton at the 4th leaf stage. The TMS glyphosate formulation, the trimethylsulfonium iodide, and in combination with technical glyphosate acid caused a significant reduction in the photosynthetic rates of Round-up-Ready™ cotton (McGraw et al 2001). The symptomology observed was similar to that observed under field conditions. These studies indicated that there was an alternate mode of action of TMS in Round-up-

Ready™ cotton and that the TMS salt itself was phytotoxic to cotton. The symptomology and rapid reduction in photosynthesis rates further suggested that the salt may be a photosynthetically active compound. Therefore, studies were conducted on ion leakage and chlorophyll florescence to further characterize the activity of trimethylsulfonium salt of glyphosate on Round-up-Ready™ cotton.

Material and Methods

General Procedures

Round-up Ready™ cotton (655 BR) Bollgard RR[2] seeds were planted in potting soil (Metro-mix 200)[2] and placed under greenhouse conditions at Gainesville, Florida. The plants reached the 3-4 leaf stage after 2- 3 weeks, at which time leaves were harvested for experimental use. Plant material for all experiments consisted of 0.7 cm^2 leaf discs. After cutting leaf discs were placed in distilled water for approximately one hour to allow callus formation.

Chemicals

Commercial grade trimethylsulfonium formulation of glyphosate was obtained from Syngenta Crop Protection, Inc.[3] Gramicidin, 2, 4 DNP (2, 4 dinitrophenol), paraquat (1, 1'-dimethyl-44'-bipyridinium dichloride) and diuron (3-(3, 4-dichlorophenyl)-11-dimethylurea) were obtained from Sigma Chemicals Inc.[4] Due to low water solubility gramicidin and diuron were dissolved initially in small volume of ethanol and then diluted to required concentrations with distilled water. Final ethanol concentrations were 1-2 % (v/v) and did not affect the leaf tissues. Trimethylsulfonium salt of glyphosate, 2, 4

[2] Delta and Pineland, Scott, MS 38772

[3] Syngenta Crop Protection, Greensboro, NC 27049

[4] Sigma Chemicals Co., West Chester, PA 19381

DNP and paraquat concentrations were made by directly by dissolving in distilled water. Unless otherwise noted, an experiment unit consisted of one 20 ml scintillation vial containing 8 leaf discs and 10 ml of treatment solution.

Rate Studies

To calculate an I_{50} concentration of trimethylsulfonium salt of glyphosate on Round-up Ready™ cotton, rate studies were performed under both light and dark conditions. Ion leakage was measured as percent conductivity (μmhos) over time as a function of concentration. For light experiments, vials were placed under continuous light (80- 120 $\mu mol.m^{-2}.s^{-1}$) at 21 C in a water bath. Concentrations tested were 10, 100, 250, 500, 750, and 1000 μM with a distilled water control. Conductivity was measured initially and 12, 24, 36, 48, 72 and 96 hours after initial exposure. For dark experiments, vials were placed on a shaker bath in the dark at 25 C. Treatment parameters were identical to light studies except conductivity measurements were stopped at 96 hours.

Total conductivity was obtained by freezing and thawing the solutions twice to release all ions. Data are presented as percent conductivity derived from the following equation:

% Conductivity = ((measured – initial)/ (total – initial))* 100

where measured equaled the amount of conductivity at the time of measurement.

Comparative Studies

The activity of trimethylsulfonium salt of glyphosate was compared to compounds with known modes of action to gain better understanding of the mechanism of trimesium salt of glyphosate on Round-up Ready™ cotton. Treatments consisted of trimesium salt of glyphosate at 500 μM (based on I_{50} calculations from rate studies), diuron (100 μM), 2,4 DNP (50 μM), gramicidin (10 μM) and paraquat (10 μM). These studies were

conducted under both light and dark conditions for a total of 96 hours. Experimental conditions were the same as previously described for the rate study.

Fluorescence

Twenty leaf discs were placed in 25ml erlenmeyer flasks containing 10 ml of treatment solution and vacuum infiltrated. Treatments consisted of trimesium salt of glyphosate at 500 μM (based on $I_{50.}$ calculations from rate studies), diuron (100 μM), 2,4 DNP (50 μM), gramicidin (10 μM) and paraquat (10 μM). Leaf discs were placed under continuous light (120 μmol.m-2.s-1) at 21 C in a water bath. Fluorescence[5] was measured at 30, 60, 90, 150, and 210 minutes. Treated leaf discs were dark-equilibrated for 10 minutes prior to chlorophyll 'a' fluorescence determination and initial, peak and terminal fluorescence measurements were taken. Terminal fluorescence was measured after 50 seconds with a 1.0 second gain between initial and peak measurements. Data are presented as the ratio of peak to terminal fluorescence derived from the following equation:

Peak / terminal ratio = (peak – initial)/ (terminal – initial)

Results are means of six replications.

Statistics

Unless otherwise noted, all treatments contained a minimum of 4 replications and studies were conducted twice. Data was subjected to analysis of variance (ANOVA) to test for interactions and treatment effects ($P < 0.05$). Data for ion leakage was pooled experiments. Results for the studies are presented with standard errors of the mean. There

[5] Plant Productivity Fluorometer, Model SF20, Richard Brancker Research, Ottawa, Ontario, Canada.

was no significant interaction between experiments for all studies, therefore data was pooled for both experiments.

Results and Discussions

Rate Studies

All concentrations caused > 30% leakage after 12 hours with nearly all causing > 80% leakage at 72 hours in light (Figure 3-1). Slightly greater (55-70%) leakage was caused by 500, 750 and 1000 μM after 12 hours (Table 3-1). In dark, all concentrations showed > 30% leakage after 24 hours, with higher concentrations showing higher levels of leakage (Figure 3-2). All the concentrations caused (≥ 25%) after 12 hours of initial exposure (Table 3-2) and showed (≥ 60%) leakage after 36 hours. Overall higher ion leakage values were observed in light than in dark. A calculated I_{50} value at 24 hours was 500 μM, and this was based on the data from light studies.

Comparison Studies

TMS formulation of glyphosate caused nearly 60% ion leakage after 12 hours of initial exposure under light conditions, which was similar to that of paraquat and diuron (Figure 3-3). Conductivity caused by TMS formulation was 80% after 24 hours of initial exposure (Table 3-3), (Table 3-1). Ion leakage expressed in (%) conductivity when exposed to different concentrations of trimethylsulfonium salt of glyphosate over time (hours) in light. Paraquat also caused rapid ion leakage, producing more than 50% total conductivity after 12 hours of initial exposure. However, the effect of this compound did not increase over the treatment period. Trimethylsulfonium salt of glyphosate and diuron produced nearly 100% conductivity after 96 hours of exposure. Gramicidin caused a minimal increase in conductivity (≤ 25%) after 48 hours, under light conditions, with only a slight increase in conductivity over the treatment period. Following exposure

under light conditions, 2, 4 DNP caused gradual increase in conductivity from (20% - 60% over 12 hours to 96 hours).

In the dark, ion leakage of trimethylsulfonium salt of glyphosate was highest at 65% after 36 hours. Gramicidin caused more rapid ion leakage in dark than in light causing almost 70 % leakage after 72 hours (Figure 3-4).

The effect of diuron and paraquat closely mirrored that of gramicidin in the dark, both of them causing nearly 50% conductivity after 48 hours. The effect of paraquat was markedly reduced in the dark from that produced in the light (Table 3-4). Paraquat caused 20% leakage after 24 hours which gradually increased to 60% after 96 hours of initial exposure in dark. 2, 4 DNP produced almost similar results in dark compared to that of light, but caused little less leakage in dark initially, as compared to that in light. Conductivity caused by 2, 4 DNP increased from 20% to 50% over a period of 36 hours to 48 hours. The effect of TMS formulation of glyphosate was markedly reduced by causing conductivity over the treatment period in the dark. The effect of TMS formulation of glyphosate was much more rapid under light conditions compared to those observed in the dark regime and resulted in higher percent conductivity values (nearly 100%) after 72 hours of treatment (Figure 3-3). Gramicidin caused similar leakage under dark conditions with less than 50% conductivity, which slightly increased over the treatment period. The leakage caused by 2, 4 DNP in dark was slightly higher, as compared to that in light (Table 3-4). Paraquat caused less conductivity under dark conditions with nearly 50% conductivity observed after 48 hours. Diuron caused more ion leakage under light conditions, causing (>90%) leakage over 72 hours.

2, 4 DNP and paraquat are known to affect both photosynthesis and respiration, injuring the cells through two independent mechanisms. 2, 4 DNP is an uncoupler of oxidative phosphorylation. Adenosine tri-phosphate (ATP) is a high-energy phosphate compound generated inside mitochondria that is required for energy dependent biological activities. The mitochondria generate ATP by generating a proton gradient with oxygen as final electron acceptor. 2, 4 DNP uncouples the membrane responsible for the proton gradient, resulting in loss of respiration and overall cell death. 2, 4-DNP also inhibits the light reactions of photosynthesis by blocking electron flow in photosystem II. The key to the mechanism of action of paraquat is the ability to form free paraquat radical by reduction and subsequent auto oxidation to yield the original ion and radical oxygen. Required for this reaction is the reducing power to convert the paraquat ion to paraquat radical and this is supplied by the photosynthetic apparatus; however, reducing power from respiration can also be utilized. In light both of these compounds cause injury through disruption of photosynthesis (as evidenced by change of fluorescence) and respiration, whereas in the dark only respiration is effected. Leakage caused by paraquat was much greater in light compared to dark primarily due to the formation of membrane disrupting radical oxygen species. Although disruption of respiration did probably occur, the impact of paraquat is much greater in the light. The opposite occurred from 2, 4-DNP where greater leakage was observed in the dark, especially after 144 hours. This indicates 2, 4-DNP is a more potent respiratory inhibitor than photosynthetic inhibitor. Although some oxidative stress is induced by inhibition or diversion of oxidative electron flow, the major cause of membrane disruption is the collapse of the membrane gradient due to lack of energy.

Diuron is a photosynthetic inhibitor that acts by blocking electron transport; more specifically by binding to the Q_B-binding niche on the D1 protein of photosystem II. This stops the production of ATP and $NADPH_2$. The inability to transfer energy promotes the formation of triplet chlorophyll and singlet oxygen, which induces lipid peroxidation and in leaky membranes. This causes cells to disintegrate rapidly. This compound has no effect on respiration and this is reflected in the low percent conductivity values in dark. Trimethylsulfonium salt appears to be acting similarly to diuron and paraquat in the dark; the decrease in the activity in the dark conditions correlates with compounds that only inhibit photosynthesis. The effect is less under dark conditions because photosynthesis would not occur in absence of light, thus diminishing activity. Gramicidin directly affects respiration but has little influence on photosynthesis. This compound also caused greater ion leakage in the dark.

Fluorescence

Trimethylsulfonium salt of glyphosate, diuron and paraquat decreased the peak / terminal ratios, relative to distilled water control (Figure 2-5) indicating an increase in chlorophyll ‘a’ fluorescence at 30, 60, 90, 150 and 270 minutes after treatment. 2, 4 DNP and gramicidin did not affect fluorescence until 90 minutes after treatment, probably due to indirect effects caused by membrane disruption (Table 3-5).

Chlorophyll ‘a’ fluorescence is a direct measure of light reaction efficiency where chlorophyll molecules re-radiate ‘excess’ absorbed light energy as fluorescence (Lawlor 1987) . Chlorophyll fluorescence is usually measured through the ratio of peak to terminal fluorescence. In normal light reactions, light is absorbed by chlorophylls and other pigments and transmitted to reaction centers where light energy is converted to chemical energy through the donation of electrons.

However, not all of the energy absorbed by chlorophyll molecules can be utilized and some is re-radiated as fluorescence. Normal fluorescence values for the ratio of peak to terminal for my study ranged from 8-12. This ratio indicates the ability of the plant to utilize light energy with higher ratios corresponding to more efficient light use, while low ratios (near 1.00) indicate that most of the energy is being lost to fluorescence.

Paraquat also produced low ratios, but this was probably due to the degradation of the photosynthetic apparatus by oxygen radicals.

Diuron produced characteristically low ratios over time, which is characteristic of compounds that block electron flow in photosystem II. Chlorophyll molecules continue to absorb light energy, and must re-radiate most of this energy as fluorescence to avoid photo-oxidation (Fuerst and Norman, 1991). The plants treated with these herbicides produce quantities of membrane-damaging triplet chlorophyll, singlet oxygen ($^1O_2$), superoxide (O_2^-), and hydrogen peroxide (H_2O_2).These radicals cause lipid peroxidation and subsequently destroy membrane integrity (Fuerst and Norman, 1991).

Gramicidin, an ionophore, which causes proton leakage from both chloroplast and mitochondrial membranes, also lowered peak / terminal ratio in conjunction with significant ion leakage. Gramicidin directly effects respiration but has little influence on photosynthesis. Therefore the effect observed on fluorescence is probably an indirect effect. Due to increased ion leakage and the disruption of photosynthetic apparatus and elevated chlorophyll fluorescence, 2, 4 DNP behaved similarly to gramicidin. Trimethylsulfonium formulation of glyphosate decreased the peak / terminal ratio, which could be because of the blocking of electron flow in the photosystem, as seen in the case of diuron and 2, 4-DNP. Collectively this data suggests that trimethylsulfonium

formulation of glyphosate effect photosynthesis in Round-up Ready™ cotton. Greater and more rapid ion leakage in the light suggests that the activity of TMS formulation of glyphosate on Round-up Ready™ cotton is light dependant and probably due to photosynthetic inhibition.

Table 3-1. Ion leakage expressed as (%) conductivity when exposed to different concentrations of trimethylsulfonium salt of glyphosate over time (hours) in light.

	Time (hours)					
Treatments	12	24	36	48	72	96
0 µM	6.2[1]	10.9	20.0	26.0	35.5	36.1
10 µM	33.3	39.0	55.8	58.0	87.4	87.4
100 µM	40.7	42.5	52.6	53.8	91.5	89.5
250 µM	44.0	33.3	49.4	57.2	93.9	88.5
500 µM	54.6	64.6	54.2	58.5	85.9	83.4
750 µM	58.0	88.3	75.2	58.2	88.7	78.0
1000 µM	69.2	65.2	58.4	51.5	55.8	37.7

[1]Means of 8 replications.

Table 3-2. Ion leakage expressed as (%) conductivity when exposed to different concentrations of trimethylsulfonium salt of glyphosate over time (hours) in dark.

	Time (hours)					
Treatments	12	24	36	48	72	96
0 µM	4.8[1]	7.8	19.8	13.5	18.6	17.2
10 µM	16.0	33.6	60.0	57.4	69.9	73.2
100 µM	12.4	34.8	72.3	35.0	47.6	63.3
250 µM	13.9	37.8	60.0	33.6	44.1	42.1
500 µM	17.9	45.2	89.0	43.2	40.7	68.4
750 µM	22.3	60.7	73.5	38.8	26.0	47.8
1000 µM	18.9	70.5	65.0	59.8	34.0	45.2

[1]Means of 8 replications.

Table 3-3. The effect of diuron, 2, 4 DNP, gramicidin, paraquat and trimethylsulfonium salt of glyphosate on ion leakage in light.

	Time (hours)					
Treatments	12	24	36	48	72	96
Water (0 µM)	14.1[1]	19.8	24.1	33.3	39.8	45.4
2,4 DNP (50 µM)	23.0	29.1	33.3	43.4	47.6	56.0
Gramicidin (10 µM)	10.6	15.4	21.0	25.0	29.6	44.6
Paraquat (10 µM)	53.4	56.1	59.0	59.6	62.3	37.0
Diuron (100 µM)	46.6	54.3	66.9	66.6	80.7	100.0
TMS (500 µm)	58.6	70.6	62.2	64.5	95.9	93.3

[1]Means of 8 replications.

Table 3-4. The effect of diuron, 2, 4 DNP, gramicidin, paraquat and trimethylsulfonium salt of glyphosate on ion leakage in dark.

	Time (hours)					
Treatments	12	24	36	48	72	96
Water (0 µM)	11.5[1]	17.4	21.2	24.9	29.4	40.0
2,4 DNP (50 µM)	11.0	16.6	24.4	50.5	55.0	64.2
Gramicidin (10 µM)	14.0	22.2	37.5	50.2	61.7	66.0
Paraquat (10 µM)	9.7	15.8	41.4	50.0	54.4	61.3
Diuron (100 µM)	10.2	24.6	39.5	48.8	64.7	72.2
TMS (500 µm)	20.9	30.8	66	47.6	40.1	38.1

[1]Means of 8 replications.

Table 3-5. The effect of diuron, 2, 4 DNP, gramicidin, paraquat and trimethylsulfonium salt of glyphosate on chlorophyll a fluorescence in Round-up Ready™ cotton leaf discs.

	Time (hours)				
Treatments	30	60	90	150	270
Water (0 µM)	3.7[1]	6.1	6.7	9.1	7.4
2,4 DNP (50 µM)	2.6	1.1	1.4	2.4	2.0
Gramicidin (10 µM)	5.9	6.0	3.4	4.5	1.6
Paraquat (10 µM)	7.2	3.8	5.1	13	11.7
Diuron (100 µM)	1.7	2.2	3.5	2.0	1.2
TMS (500 µm)	1.8	3.2	3.2	1.5	0.5

[1] Peak/ Terminal Ratio

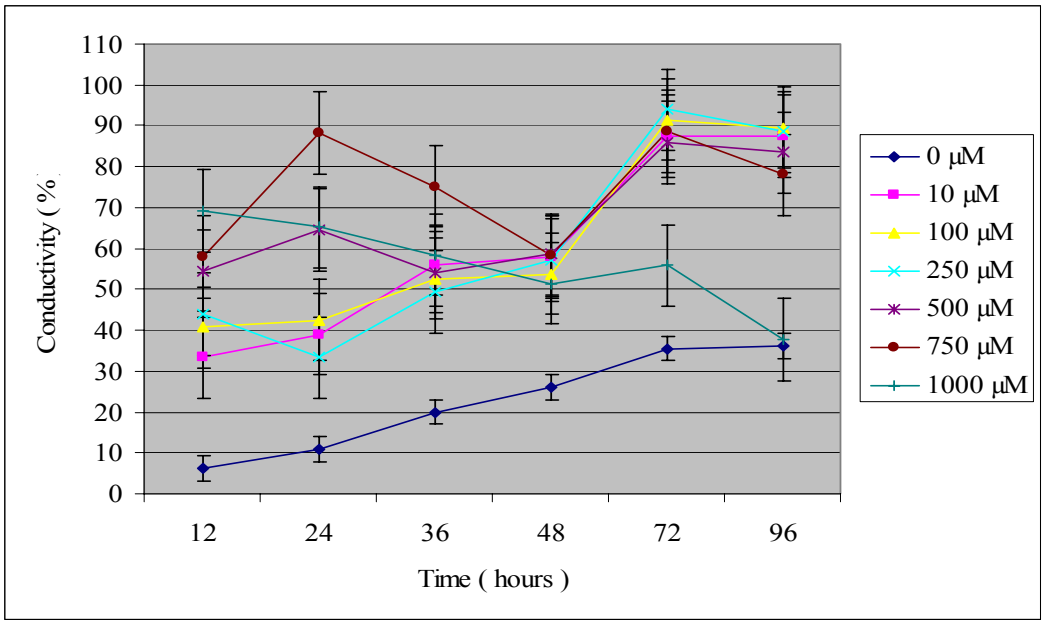

Figure 3-1. Ion leakage expressed as % conductivity when exposed to different concentrations of trimethylsulfonium salt of glyphosate over time (hours) in light.

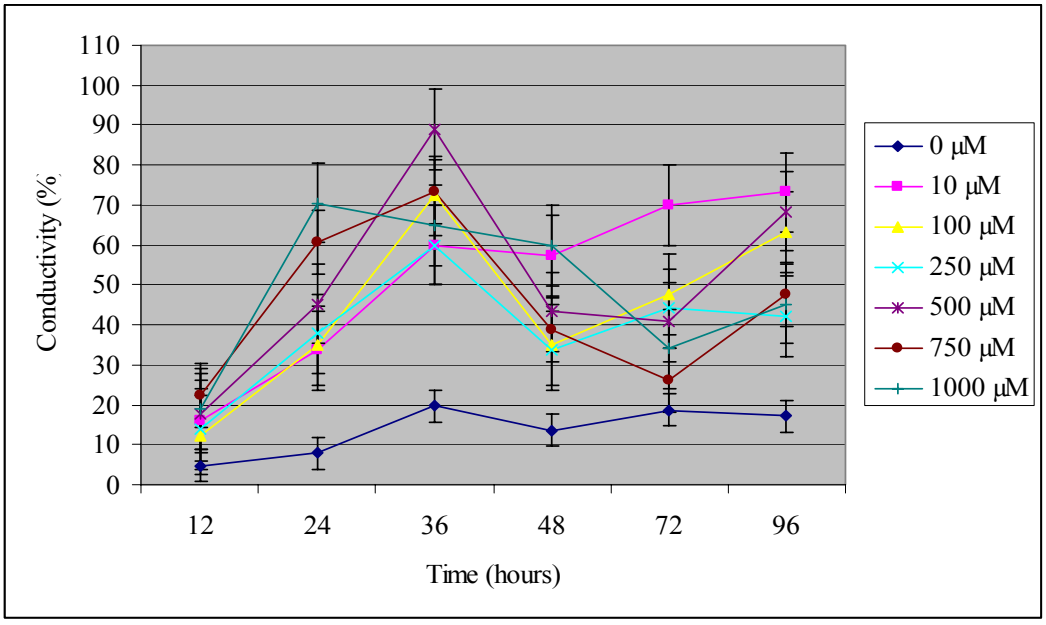

Figure 3-2. Ion leakage expressed as (%) conductivity when exposed to different concentrations of trimethylsulfonium salt of glyphosate over time (hours) in dark.

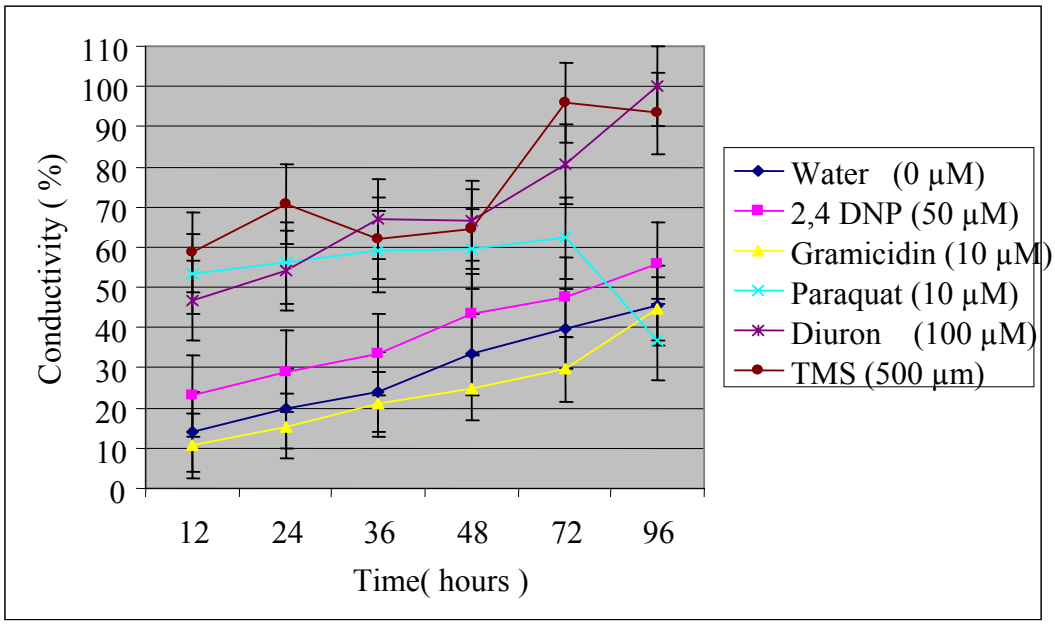

Figure 3-3. The effect of diuron, 2, 4 DNP, gramicidin, paraquat and trimethylsulfonium salt of glyphosate on ion leakage in light.

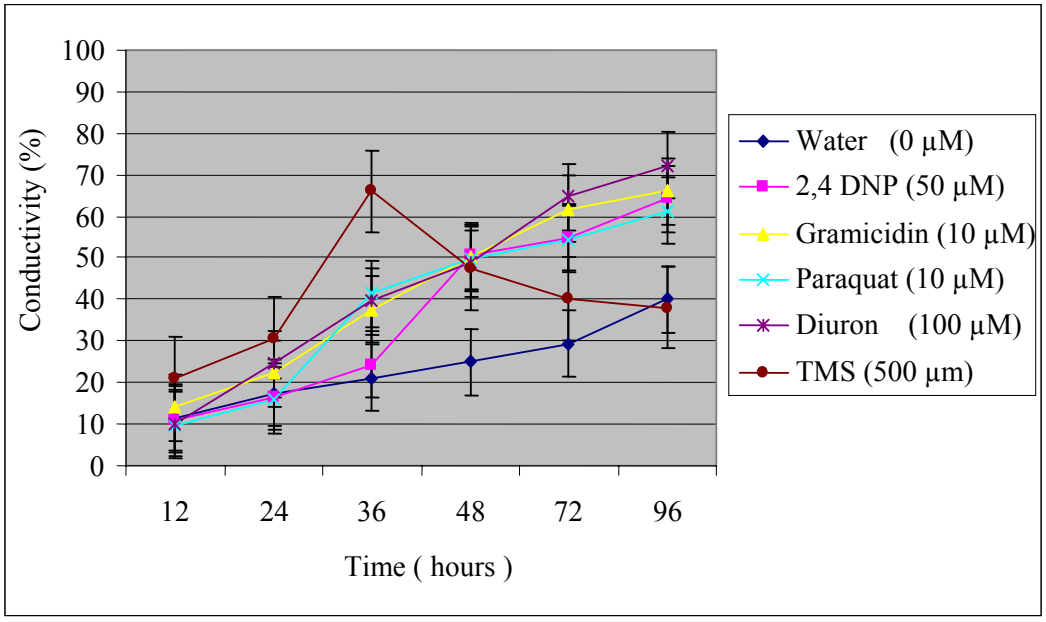

Figure 3-4. The effect of diuron, 2, 4 DNP, gramicidin, paraquat and trimethylsulfonium salt of glyphosate on ion leakage in dark.

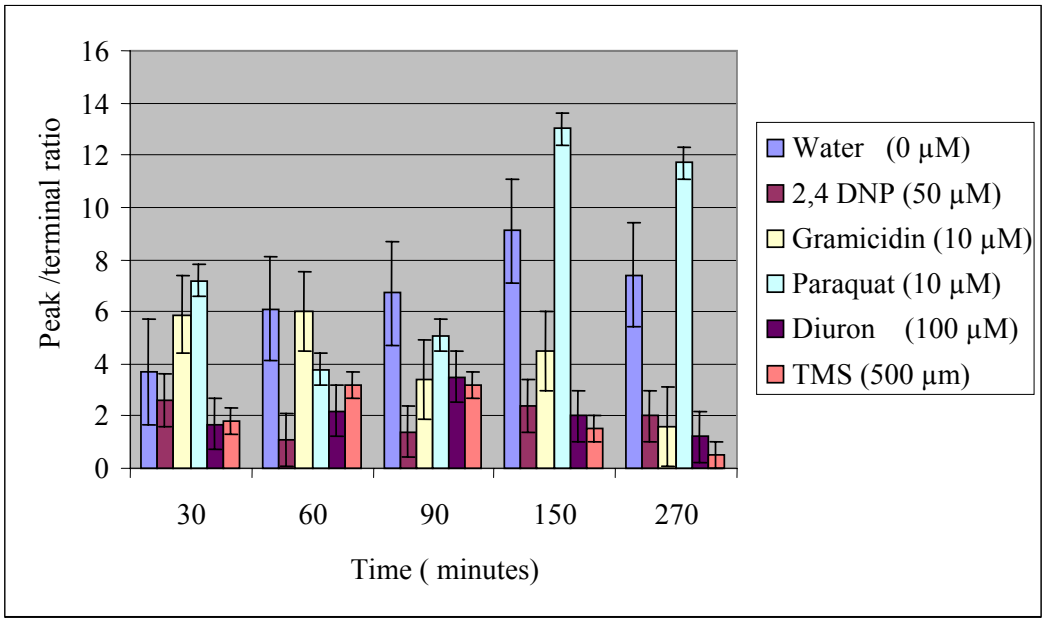

Figure 3-5. The effect of diuron, 2, 4 DNP, gramicidin, paraquat and trimethylsulfonium salt of glyphosate on chlorophyll a fluorescence in round-up ready cotton leaf discs.

CHAPTER 4
SUMMARY

Characterization of the Activity of Fluazifop on Bristly Starbur

Bristly Starbur (*Acanthospermum hispidum DC.*) is categorized as one of the main weeds in many crop fields throughout a broad region from tropical to temperate zones (Walker et al. 1989; Panizzi and Rossi 1991). This weed is a major problem in corn, peanuts and soybeans in Florida because it directly competes with moisture light and nutrients in the soil (Hall et al. 1991). This weed also increases the cost of production by interfering in harvesting, since it continues to grow until a killing frost (Vester 1974). Fluazifop is the active ingredient in several herbicides which are registered for use at 0.05- 0.21 kg ai/ha for control of annual and perennial grasses. Typical symptomology of fluazifop includes immediate cessation of growth after application, followed by leaf chlorosis within one to three weeks. Fluazifop acts by inhibiting acetyl-CoA carboxylase (ACCase) activity, which is the initial step in fatty acid synthesis. This leads to the inhibition of lipid biosynthesis which causes a cessation of growth and death occurs over a period of 14-21 days. Although this mode-of-action has been well documented in grasses, an alternative mode-of-action has been observed on the broad leaf species bristly starbur (*Acanthospermum hispidum DC)*. In previous studies under greenhouse conditions, fluazifop was observed to cause complete death of starbur at 0.25 lb ai /A. Moreover this injury occurred in 3-5 days, atypical of the reported mode-of-action on grassy weeds. Additional research suggests that fluazifop activity occurs at the membrane level in starbur, possibly through lipid peroxidation. To further characterize

fluazifop activity on bristly starbur, ion leakage and chlorophyll fluorescence studies were performed.

All assays utilized 0.7 cm diameter leaf discs obtained from greenhouse grown starbur and all experiments were conducted twice with a minimum of three replications. There was differential response of fluazifop rate under light and dark conditions. The rate which caused 50% ion leakage was significantly higher in light compared to dark. There was a decreasing affect of rate as exposure time increased, with >90% ion leakage occurring after 96 hours exposure time. Ion leakage caused by fluazifop (600 µmol) was also compared to compounds with known mechanisms of action. These included paraquat, diuron, 2, 4-dinitrophenol and the proton ionophore, gramicidin. Fluazifop caused more than 60% ion leakage after 96 hours under light conditions, which was similar to that observed from diuron. In contrast, fluazifop appears to be acting similarly to 2, 4-DNP and gramicidin in the dark; causing >95% ion leakage only after 24 hours under dark conditions Gramicidin directly affects respiration but has little influence on photosynthesis. This compound also caused greater ion leakage in the dark. Percent conductivity caused by fluazifop, paraquat and diuron increased over time with paraquat causing nearly 95% conductivity after 96 hours under continuous light. The effect of fluazifop was much more rapid under dark conditions compared to those observed in the light regime.

Chlorophyll fluorescence studies were also performed using comparative compounds that have known effects on photosynthesis. Fluazifop, diuron and paraquat decreased the peak / terminal ratios, relative to distilled water control and thereby

showing an increase in chlorophyll fluorescence, behaving similarly to the photosynthetic inhibitors diuron and paraquat.

These results of these studies indicate the mechanism of action of fluazifop is not light dependent due to the increased activity under dark conditions. This suggests some level membrane activity, similar to previous research, but results indicate a more direct impact possibly membrane uncoupling.

Characterization of the Activity of Touchdown on Round-up Ready Cotton

Poor weed control has been cited as one of the greatest limitation to successful cotton (*Gossypium hirustum L.*) production (McWhorter and Jordon 1985). Glyphosate herbicide has been primarily used in cropping systems as a burndown herbicide applied in minimum tillage operations before the introduction of glyphosate resistant crops. Glyphosate effectively controls many dicotyledonous and monocotyledonous weeds common to agronomic crops. Symptomology includes inhibited growth soon after application followed by general foliar chlorosis and necrosis within 7-21 days. Glyhosate acts by inhibiting 5-enolpyruvylshikimate 3-phosphate (EPSP) synthase (Amrhein et al. 1980) in the shikimic acid pathway, which leads to several metabolic disturbances due to depletion of aromatic amino acids tryptophan, tyrosine, and phenylalanine, including the arrest of protein production and prevention of secondary product formation (Franz, Mao and Sikorski 1997).The deregulation of the shikimate pathway also leads to general metabolic disruption (Duke et al 2003, 1988).

Round-up Ready^{TM} cotton is resistant to vegetative injury from glyphosate herbicide, but detrimental effects on reproductive development may occur if glyphosate is applied beyond the four-leaf stage.

The trimethylsulfonium (TMS) salt of glyphosate has become a popular alternative to the IPA salt of glyphosate since 1999. Similar toxicities and weed control have been reported with both formulations. There were no differences between formulations because both formulations were ionized to the same active glyphosate acid. However, trimethylsulfonium salts may affect plant growth and research has demonstrated that phytotoxic effects have been observed on several plant species. The difference in inflated duckweed (*Lemna gibba L.*) control between the TMS and IPA formulation of glyphosate was caused by the TMS portion of the formulation (Sùrensen and Gregersen 1999), suggested that the lethal mechanism between glyphosate and trimethylsulfonium salts of glyphosate may be different.

Several studies have indicated that there was an alternate mode of action of TMS in Round-up-Ready^{TM} cotton and that the trimesium salt itself was phytotoxic to cotton. The symptomology and rapid reduction in photosynthesis rates further suggested that the salt may be a photosynthetically active compound. Therefore, studies were conducted on ion leakage and chlorophyll florescence to further characterize the activity of trimethylsulfonium salt of glyphosate on Round-up-Ready^{TM} cotton.

All assays utilized 0.7 cm diameter leaf discs obtained from greenhouse grown Round-up-Ready^{TM} cotton and all experiments were conducted twice with a minimum of three replications. There was differential response of trimethylsulfonium salt of glyphosate under light and dark conditions. All concentrations caused > 30% leakage after 12 hours with nearly all causing > 80% leakage at 72 hours in light. In dark, all concentrations showed > 30% leakage after 24 hours, with higher concentrations showing higher levels of leakage. Overall higher ion leakage values were observed in light than in

dark. Ion leakage caused by I_{50} dose (500 µmol) trimethylsulfonium salt of glyphosate was also compared to compounds with known mechanisms of action. These included paraquat, diuron, 2, 4-dinitrophenol and the proton ionophore, gramicidin. Trimethylsulfonium salt appears to be acting similarly to diuron and paraquat in the dark; causing nearly 65 % ion leakage after 36 hours of initial exposure under dark conditions. In contrast, the effect of TMS formulation of glyphosate was much more rapid under light conditions, resulting in higher percent conductivity values (nearly 100%) after 72 hours of treatment. The effect of TMS formulation of glyphosate was markedly reduced by causing conductivity after 12 hours, reaching 70 % after 36 hours in the dark.

Gramicidin caused similar leakage under dark conditions with less than 50% conductivity, which slightly increased over the treatment period. The leakage caused by 2, 4 DNP in dark was slightly higher, as compared to that in light. trimethylsulfonium salt appears to be acting similarly to diuron and paraquat in the dark; the decrease in the activity in the dark conditions correlates with compounds that only inhibit photosynthesis. Gramicidin also caused greater ion leakage in the dark.

Chlorophyll fluorescence studies were also performed using comparative compounds that have known effects on photosynthesis. trimethylsulfonium salt of glyphosate, diuron and paraquat decreased the peak / terminal ratios, relative to distilled water control indicating an increase in chlorophyll ‘a’ fluorescence at 30, 60, 90, 150 and 270 minutes after treatment. 2, 4 DNP and gramicidin did not affect fluorescence until 90 minutes after treatment, probably due to indirect effects caused by membrane disruption. Paraquat also produced low ratios, but this was probably due to the degradation of the photosynthetic apparatus by oxygen radicals. Diuron produced characteristically low

ratios over time. Gramicidin also lowered peak / terminal ratio in conjunction with significant ion leakage, which was an indirect effect. Due to increased ion leakage and the disruption of photosynthetic apparatus and elevated chlorophyll fluorescence, 2, 4 DNP behaved similarly to gramicidin. Trimethylsulfonium formulation of glyphosate decreased the peak / terminal ratio, which could be because of the blocking of electron flow in the photosystem, as seen in the case of diuron and 2, 4-DNP.

Collectively this data suggests that trimethylsulfonium formulation of glyphosate effect photosynthesis in Round-up Ready^{TM} cotton. Greater and more rapid ion leakage in the light suggests that the activity of TMS formulation of glyphosate on Round-up Ready^{TM} cotton is light dependant and probably due to photosynthetic inhibition.

APPENDIX
HERBICIDE COMMON AND CHEMICAL NAME

. Herbicide common and chemical names.

Common Name	Chemical Name
Fluazifop	[Butyl 2-(4-((5-(trifluoromethyl)-2-pyridinyl) oxy) phenoxy) propanoate)]
2, 4 DNP	[2, 4 dinitro phenol]
Diuron	[3-(3, 4-Dichlorophenyl)-11-dimethylurea]
Paraquat	[1, 1'-Dimethyl-44'-bipyridinium dichloride]
Gramicidin	gA
Touchdown	[N-(Phosphonomethyl)glycine, in the form of trimethylsulfonium salt]

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BIOGRAPHICAL SKETCH

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