1. LITERATURE REVIEW

1.1 INTRODUCTION

The development of herbicide resistant crops offers producers many more options for weed control systems. These crops allow non-selective, broad-range herbicides to be used as selective herbicides, effectively controlling a wider range of weed species than a selective herbicide would, and at the same time, not injuring the crop. Most of the herbicide resistant crops available today are made resistant by the addition of a gene coding for an enzyme which detoxifies the herbicide, or a gene which codes for an altered form of an enzyme targeted by the herbicide. The herbicide fails to bind to the altered enzyme, and the enzyme preserves its function in the plant.

Glufosinate, a rapid-acting postemergence herbicide is being incorporated into field cropping systems with the use of glufosinate tolerant, or Liberty-Link® crops (Bertges et al., 1994). Glufosinate tolerant, Liberty-Link® corn (*Zea mays* L.) was approved for commercialization in 1997, and Liberty-Link® soybean varieties are currently under development. The non-selective herbicide glufosinate can be applied safely to Liberty-Link® soybeans offering good weed control. Glufosinate tolerance is conferred to plants by incorporation of either the *pat* (phosphinothricin-acetyltranserfase) or the *bar* (bialaphos resistance) genes which code for enzymes that inactivate glufosinate by acetylation (Mullner et al., 1993).

Another line of transgenic herbicide resistant soybeans that has been commercialized recently, is a Roundup-Ready® soybean. Roundup-Ready® soybeans contain a glyphosate insensitive 5-enolpyruvylshikimate-3-phosphate synthase (EPSP synthase) gene introduced from *Agrobacterium* sp. conferring tolerance to the non-selective herbicide glyphosate (Padgette et al., 1995). Roundup-Ready® soybeans were commercialized in 1996 and are now accounting for between 50-60% of the soybeans grown in the U.S (Filajdic, 1999).

As these crops are very new on the market, investigation of their performance under various environmental conditions and in weed control systems is needed.

1.2 MODE OF ACTION OF GLUFOSINATE

Glufosinate is a non-selective post emergence herbicide that controls weeds by irreversibly inhibiting the enzyme glutamine synthetase (Wild and Manderscheid, 1984). Glutamine synthetase (E.C. 6.3.1.2) incorporates glutamate and ammonia to form the amino acid glutamine. The enzyme has different isoforms contained in leaves and roots. The root isoform of glutamine synthetase was shown to have greater sensitivity to phosphinothricin (glufosinate) than the chloroplast isoform in both *Sinapis alba* and *Triticum aestivum* (Wild and Manderscheid 1984; Manderscheid and Wild, 1986). Inhibition of this enzyme causes a large buildup of ammonia in plant cells (Wild and Manderscheid, 1984; Tachibana et al., 1986), as well as depleting the plant of crucial amino acids, and inhibiting photosynthesis. The accumulation of ammonia in plant cells due to glutamine synthetase inhibition was initially thought to cause the observed phytotoxcicity. However, more recent studies show that ammonia accumulation does not appear to be the primary cause of cell death after glufosinate treatment. Krieg et al. (1990), found that application of up to 40 times the standard 10 mM level of ammonium nitrate used in the study to culture media of *Medicago sativa* cultures had no effect on callus growth even with a 27 fold increase in endogenous ammonia. This would suggest that cellular accumulation of ammonia alone may not cause the drastic phytotoxicity observed in plant cells following glufosinate application.

Photosynthesis is also rapidly inhibited by glufosinate under atmospheric levels of O_2, but not under non-photorespiratory O_2 conditions (Sauer et al., 1987; Wild et al., 1987; Lacuesta et al., 1992). Photosynthesis inhibition is not due to ammonia accumulation, but instead to a decrease in the concentration of the amino acids glutamine, glutamate, aspartate, alanine, glycine and serine. Addition of glutamate and glutamine to glufosinate treated plant tissue increased the content of these amino acids over those treatments with glufosinate alone (Wild and Wendler,1991; Downs et al., 1994). Three subsequent affected reactions may cause inhibition of photosynthesis (Sauer et al., 1987). First, inhibition of protein biosynthesis occurs due to lack of glutamine production.

Secondly, a toxic accumulation of glyoxylate occurs in the photorespiratory cycle. Glyoxylate inhbits RuBP-carboxylate and carbon dioxide fixation. Finally, a deficiency of intermediates of the Calvin cycle occurs due to the interruption of photorespiration (Sauer et al., 1987).

Injury symptoms caused by glufosinate include rapid chlorosis of treated tissue, followed by necrosis and ultimate death of treated plants within a few days. Bellinder et al. (1987) observed rupture and contortion of interveinal mesophyll cells with concomitant disorganization of bundle sheath cells. Rapid epidermal collapse also occurred in redroot pigweed (*Amaranthus retroflexus* L.). Ullrich et al. (1990), found that the addition of phosphinothricin caused a primary electrical depolarization of the plasmalemma without recovery, also causing an increase in extracellular pH due to its uptake as a strongly dissociated anion. Phosphinothricin also induced a continuous K^+ release. It is thought to be taken up by a proton co-transport mechanism, using the same carrier as neutral and acidic amino acids. Accumulated ammonium and direct interference function to uncouple the glutamate/H^+ co-transport.

Thus, the inhibition of the glutamine synthetase enzyme in plants is manifested by ammonia accumulation, inhibition of amino acid synthesis, inhibition of photosynthesis, and severe damage to plant tissues which ultimately result in death of the treated plant.

1.2.1 Discovery of glufosinate and resistance genes

L-Phosphinothricin (Bayer et al., 1972), is the herbicidal component of bialaphos (Kondo et al., 1973), a natural tri-peptide produced by at least two *Streptomyces hygroscopicus* species as an extracellular product. Glufosinate is the name for the chemically synthesized racemic mixture of D, L-phosphinothricin. Apart from isolating the herbicide bialaphos from *Streptomyces hygroscopicus*, researchers have isolated two genes (*pat* and *bar*) whose protein products detoxify glufosinate by acetylation. Phosphinothricin acetyl-transferase, *pat*, and bialaphos resistance, *bar*, acetylate the free NH_2 group of phosphinothricin thus inactivating it from binding to glutamine synthetase (Thompson et al., 1987). Both *PAT* and *BAR* enzymes are similar in their ability to selectively acetylate glufosinate and not other amino acids, have comparable molecular

weights, and show immuno-cross-reactivity to their respective antisera (Wehrmann et al., 1996).

1.2.2 Absorption and translocation of glufosinate

Ullrich et al. (1990), proposed that glufosinate, because of its structural similarity to amino acids such as glutamate, is taken up by a proton co-transport mechanism, which transports neutral and acidic amino acids. The authors also reported that uptake of phosphinothricin was stimulated by light and a pH 5 or below. Absorption of ^{14}C-glufosinate has been shown to be very rapid for the first hours after treatment, but showing little increase after 24 hours (Ullrich et al., 1990; Steckel et al., 1997). It is possible that transport mechanisms are completely saturated or un-coupled by ammonia accumulation after 24 hours causing little subsequent increase in absorption.

Translocation of ^{14}C-glufosinate is limited in treated plants. Steckel et al. (1997) found that over 88% of applied ^{14}C-glufosinate remaining in the treated leaves of four weed species 72 hours after treatment. Less than 11% of the absorbed ^{14}C-glufosinate reached the roots in all species investigated (Steckel et al., 1997; Mersey et al., 1990; Haas and Muller, 1987).

Differential absorption, translocation, and metabolism of herbicides can determine the relative degree of plant sensitivity or tolerance to certain herbicides (Wanamarta and Penner, 1989). Differential tolerance of weed species to glufosinate was attributed to differential absorption and translocation among the species (Steckel et al., 1997; Mersey et al., 1990; Ridley and McNally, 1985). The most sensitive species tested, giant foxtail, absorbed 4 times as much ^{14}C-labeled glufosinate as the most tolerant species, common lambsquarters. In greenhouse studies, this increased absorption was reflected by a glufosinate I_{50} (glufosinate rate which inhibits the growth of the weed by 50%) for lambsquarters, which was 3.4 times greater than for giant foxtail. Ridley and McNally (1985) claimed a 70-fold difference in glufosinate sensitivity in weed species. These differences were not caused by differences in the degree of glutamine synthetase inhibition by glufosinate in different plants, and were thus attributed to possible differences in absorption, translocation, or metabolism of glufosinate.

1.2.3 Metabolism of the herbicide glufosinate

Glufosinate is not metabolized appreciably by treated plants. However, different metabolites of glufosinate are detected in plants transformed with the *pat* gene, than those found in plants not containing the gene. Three metabolites (4-methylphosphinico-2-oxo-butanoic acid, 3-methylphosphinico-propanoic acid, and 4-methylphosphinico-2-hydroxy-butanoic acid) were found in the non-transformed plants versus mainly one metabolite, the acetylated glufosinate, found in transformed plants. The three metabolites found in non-transformed plants were not detected in the transformed plants. This indicates that the transgene acetylation of glufosinate competes a with plant specific pathway of glufosinate metabolism. (Dröge-Laser et al., 1994; Dröge et al., 1992).

1.2.4 Crops engineered with glufosinate resistance

The *bar* and *pat* genes have been used successfully in transfering herbicide resistance to glufosinate in a number of crop plants. Cloning of the resistance genes with a number of promoters has been reported. The CaMV 35S promoter was used successfully to transform tobacco, tomato, and potato (Botterman and Leemans, 1989) and the root-specific par promoter from the hemoglobin gene from *Parasponia andersonii*, to transform tobacco (van der Hoeven et al., 1994). Field tests using plants with both promoters indicate that resistance of engineered plants exceeded that needed for tolerance to field rates of glufosinate. Safety was observed at glufosinate rates greater than 5 kg ai/ha. (Botterman and Leemans, 1989; van der Hoeven et al., 1994). Other crops that have been transformed successfully for glufosinate resistance include wheat (Vasil et al., 1992), rice (Christou et al., 1991), maize (Gordon-Kamm et al., 1990), sugarbeet (D'Halluin et al., 1992), oilseed rape (Cobb, 1992), alfalfa (Cobb, 1992), potato (De Greef et al., 1989; De Block et al., 1987), and tomato (De Block et al., 1987). Other smaller acreage crops are currently in development. Glufosinate tolerant maize and canola are already on the market in North America, and soybeans are currently under development. As of today, there have been no reports of naturally occurring, resistant plants or mutations conferring glufosinate resistance. The broad spectrum of weeds controlled, lack of resistant weeds, low toxicity, no soil residual problems, and multitude

of glufosinate resistant crops to be available, make the use of glufosinate in cropping systems a very appealing option for growers.

1. 3 MODE OF ACTION OF GLYPHOSATE

Glyphosate is a non-selective herbicide that has its primary mode of action by inhibiting the plant enzyme 5-enolpyruvoyl-shikimate-3-phosphate synthase (EPSP synthase, E.C. 2.5.1.19). This enzyme catalyzes the formation of EPSP from phosphoenolpyruvate (PEP) and shikimate 3-phosphate (S3P). Inhibition of EPSP synthase blocks the production of the important branch point intermediate, chorismate. Chorismate is required for the biosynthesis of a wide variety of aromatic plant metabolites, including the aromatic amino acids phenylalanine, tyrosine, and tryptophan. These aromatic amino acids are used as precursors for numerous secondary plant products such as anthocyanins, lignin, growth promoters, growth inhibitors, and phenolics, as well as protein production (Franz et al., 1997).

Injury symptoms following treatment with glyphosate are often slow in developing. Chlorosis is followed by necrosis and eventual plant death can take up to two weeks. The symptoms occur primarily as a result of starvation for amino acids, proteins, and secondary plant products derived from chorismate.

1.3.1 Absorption of glyphosate

Absorption of glyphosate under favorable conditions is characterized by initial fast entry, followed by a longer phase of slower absorption. The extent to which glyphosate is absorbed by plants is generally dependent on factors such as plant species, age, cuticular properties, environmental conditions, the concentration of glyphosate used, adjuvants used, and the method of application (Caseley and Coupland, 1985).

Glyphosate is a highly polar molecule with low lipophilic characteristics. The relatively rapid passage of glyphosate and its water-soluble salts through the leaf surface into the apoplast suggests that an aqueous or hydrophilic pathway through the lipophilic cuticle may be operational (Schönherr, 1979). After entering the apoplast, glyphosate

entry into plant cells was found to be much slower. Entry could be either through a passive diffusion mechanism that is not affected by pH (Gougler and Geiger, 1981), or by an endogenous transport system, possibly a phosphate carrier within the plasmalemma (Mervosh and Balke, 1981; Burton and Balke, 1988).

Environmental factors and plant growth stage also affect absorption of glyphosate. Absorption of glyphosate is usually increased by any factor that raises the water potential of the plant such as an increase in soil moisture or relative humidity (Caseley and Coupland, 1985). Also, plants grown under low light intensity generally produce less cuticular wax and are reported to absorb more glyphosate than plants grown under higher light intensity. Temperature seems to have less effect on absorption than other environmental factors (Franz et al., 1997). The influence of plant growth stage on glyphosate absorption is very species dependent. For example, barnyardgrass (*Echinochloa crus-galli* L.) seedlings 5 and 10 cm in height were no different in absorption of glyphosate (Ahmadi et al., 1980). However, quackgrass (*Elytrigia repens* L.) showed twice as much glyphosate absorption in mature plants than plants at boot stage (Davis et al., 1979).

1.3.2 Translocation of glyphosate

Glyphosate translocation is generally rapid in most plants. The rapid translocation of glyphosate to all plant parts accounts for the systemic activity of this herbicide (Franz et al., 1997). After glyphosate penetrates the plasmalemma, glyphosate can readily enter the symplast and is extensively translocated throughout the plant *via* the phloem sieve tubes. Glyphosate can also undergo translocation *via* plasmodesmata from cell to cell. Along with symplastic translocation, considerable apoplastic translocation also occurs (Jachetta et al., 1986).

Glyphosate is generally translocated in the same distribution pattern as the photoassimilates in many plants thus following a source to sink relationship (Martin and Edington, 1981; Wyrill and Burnside, 1976). Many studies indicate that glyphosate absorption and translocation do not proceed at the same rate (Franz et al., 1997).

The amount and pattern of glyphosate translocation is also dependent on plant species. Variable rates of translocation out of the treated leaf were found in five different weed species ranging from 3.5 to 21.6% (Sandberg et al., 1980).

Translocation of glyphosate is also affected by environmental factors such as light, temperature, humidity, etc. Increased light intensity enhanced the development of glyphosate injury. Studies in quackgrass found that increased light intensity caused faster translocation of ^{14}C-glyphosate to rhizomes and crowns during the first 48 hours after treatment (Coupland, 1983). Temperature effects on transport of ^{14}C-glyphosate are very species dependent. Translocation increased at higher temperatures in hemp dogbane (*Apocynum cannabinum* L.) (Schultz and Burnside, 1980) and johnsongrass (*Sorghum halepense* L.) (McWhorter et al., 1980), yet decreased in soybean from 24° to 53° C. The variability of temperature and translocation effects may be related to rhizome metabolism, and the specificity of species to optimum temperature for photosynthesis and assimilate transport (Duke, 1988).

1.3.3 Tolerance to glyphosate

The natural development of weed resistance to glyphosate has been virtually unheard of to date, even though glyphosate has been used internationally for more than two decades (Holt et al., 1993). However, recent reports from Australia have shown that a biotype of ryegrass (*Lolium rigidum* Gaud.) has developed resistance to glyphosate (Preston et al., 1999; Pratley et al., 1999; Gruys et al., 1999). Some plants in a dormant stage are tolerant to glyphosate, but this is due to a limitation in glyphosate absorption or translocation. (Robinson, 1985). The lack of development of mutations resulting in glyphosate resistance has been attributed to the short life of glyphosate in soil. This lack of naturally occurring glyphosate resistant weeds makes the use of glyphosate in Roundup-Ready® soybeans a welcome alternative to traditional herbicides, to which many weeds have developed resistance.

There are at least three synthetic methods that glyphosate resistance has been conferred onto plants.

1.3.3.1 Altered EPSP synthase

Attempts to alter the structure of the EPSP synthase enzyme in such a way that it is functional in the production of EPSP and phosphate as well as insensitive to the herbicide glyphosate have been quite intensive in the last two decades. Padgette et al. (1991), concentrated on the G101A (glycine to alanine substitution at position 101) of petunia EPSP synthase, but no resulting plants were highly glyphosate tolerant and bound the PEP substrate comparably to the wild-type EPSP synthase.

A naturally occurring EPSP synthase gene was identified from *Agrobacterium sp.* strain CP4, whose protein product had favorable glyphosate tolerance kinetic parameters such as high glyphosate tolerance and tight binding of PEP (Barry et al., 1992; Padgette et al., 1995). This gene was then cloned from *Agrobacterium sp.* strain CP4 and expressed in several crop plants including soybeans.

Soybeans carrying the altered EPSP synthase gene are already on the market and have shown to be resistant to normal field rates of glyphosate (Delanney et al., 1995).

1.3.3.2 Overproduction of EPSP synthase

Plant tolerance to glyphosate may result also from the overproduction of EPSP synthase due to gene amplification or alteration in gene transcription. Cultured carrot cells, after adaptation to 25 mM glyphosate, produced a cell line with a 52-fold increase in glyphosate tolerance and a 12-fold increase in EPSP synthase activity (Nafzinger et al., 1984). Other studies have shown similar results in other plant cell cultures (Steinrucken et al., 1986; Dyer et al., 1988). Most of these studies have demonstrated that EPSP synthase gene amplification appears to be a stable phenotype even when the selected culture is grown for a period in a glyphosate-free environment. These glyphosate mutations were selected by culturing cells in a lethal concentration of glyphosate and then selecting for survivors. All of the reported glyphosate resistant mutants that derive from this type of selection still contain the native, glyphosate sensitive EPSP synthase. Tolerance is due to the overexpression of EPSP synthase, leaving a sufficient amount of EPSP synthase that is not inhibited by glyphosate to carry on normal plant metabolism.

1.3.3.3 Metabolic detoxification

Glyphosate metabolism by plants has been reported (Coupland, 1985), however it remains unclear whether this metabolism was due to plants or to the microbes present on leaf surfaces. There are two major metabolic pathways found in microbes for glyphosate detoxification. The first involves oxidative cleavage of the nitrogen-carbon bond to yield aminomethylphophonic acid (AMPA), which can be further degraded to inorganic phosphate (Jacob et al., 1988). A second pathway involves the breaking of the phosphorus-carbon bond by a novel C-P lyase to generate sarcosine, which can be further metabolized (Dick and Quinn 1995). Commercial levels of glyphosate tolerance have been achieved in canola, tobacco, and other crops by using the glyphosate oxidoreductase enzyme isolated from *Pseudomonas* sp. strain LBr, which uses the AMPA metabolism route. (Franz et al., 1997).

Due to the large number of reports citing boll abortion and injury in Roundup-Ready cotton plants, and stem injury in Roundup-Ready soybeans (Gertz and Vencill, 1998) transformations of metabolic detoxification genes able to metabolize glyphosate may be used to replace the current altered enzyme transformants.

1.4 USE OF CHEMICAL SYNERGISTS TO INCREASE EFFICACY OF GLUFOSINATE AND GLYPHOSATE

As producers shift to no-till cultural systems for soybean production, the use of herbicide resistant transgenic soybeans is a very desirable option. Non-selective herbicides such as glufosinate or glyphosate have a high amount of activity on a broad spectrum of weeds, both annual and perennial. Shifts to no-till farming often include shifts in the weed spectrum from annual weed problems to perennial weed problems.

Glyphosate, which is translocated well in most plants controls a broad spectrum of perennial weeds by killing off top growth and preventing re-growth from underground structures.

Because of the relatively limited translocation of glufosinate in weed species, perennial weeds are not controlled effectively by glufosinate. Since glufosinate is poorly transported to roots and underground stems, even if the top growth is suppressed,

perennials are able to re-grow. Glufosinate appears to be more phloem-mobile than xylem-mobile (Shelp et al., 1992). Perhaps the efficacy of glufosinate could be improved in annual and tough perennial weeds by using additives such as chlorflurenol, 6-benzyl-aminopurine, gibberellic acid, abscisic acid, or ethephon which have been shown to increase herbicide translocation in the phloem (Devine et al., 1993). It is also possible that the use of additives such as ammonium sulfate or pelargonic acid with glufosinate or glyphosate could increase their efficacy by causing more rapid tissue desiccation.

1.4.1 Ammonium sulfate and pelargonic acid as herbicide synergists

The use of chemical synergists increases the efficacy of many herbicides. Salts such as ammonium sulfate have been reported to increase phytotoxicity of many herbicides including glyphosate (Blair, 1975; Nalewaja and Matysiak, 1993; O'Sullivan, et. al., 1981; Suwunnamek and Parker, 1975; Turner and Loader, 1975 and 1981). The reported synergism of glyphosate activity by ammonium sulfate has been attributed to its ability to overcome the formation of glyphosate salts in hard water (Nalewaja and Matysiak, 1993), and to an enhancement of herbicide absorption (Gronwald et al., 1993).

Pelargonic acid, a naturally occurring nine-carbon fatty acid in animals, causes extremely rapid and non-selective burn-down of green tissue. Pelargonic acid, sold under the trade name Scythe®, has been claimed to increase absorption of glyphosate, while concurrently causing more rapid desiccation (Savage and Zorner, 1996). Perhaps pelargonic acid could also enhance the activity of glufosinate. The safety of Liberty-Link® soybeans to these synergists would also need to be tested.

1.5 EFFECT OF TEMPERATURE ON HERBICIDE TOLERANCE

Metabolism-based resistance of plants to herbicide has been shown to be temperature sensitive. For example maize, which is normally tolerant of atrazine because of metabolism, is damaged at low temperatures. The glutathione conjugation and

hydroxylation reactions that detoxify this herbicide under normal conditions were retarded at low temperatures resulting in crop damage (Thompson et al., 1970).

Presently there is no information regarding the metabolism of glyphosate and glufosinate in transgenic varieties of herbicide resistant soybeans at variable temperatures. The enzyme encoded by the *pat* gene contained in Liberty-Link® soybeans has been shown to have very low activity at 10° C and increasing activity to 45° C (Botterman et al., 1991). Night temperatures as low as 10°-15° C are not uncommon during the soybean-growing season. Perhaps these low temperatures could cause crop injury after herbicide application by the low activity of the metabolism enzymes at these temperatures.

1.6 HERBICIDE APPLICATION FOR CONTROL OF PHYTOPATHOGENS

Phosphinothricin, the active ingredient in the herbicide glufosinate, was originally isolated from *Streptomyces* species. These bacteria produce phosphinothricin naturally as a defense mechanism against other pathogens. Transgenic rice transformed with the *pat* gene and treated with glufosinate, was less sensitive to the fungi *Magnaporthe grisea* and *Rhizoctonia solani* causing rice blast and sheath blight diseases of rice. (Tada et al., 1996; Uchimiya et al., 1993).

Glyphosate has been shown to inhibit the growth of the soil fungus *Calonectria crotalariae*, the pathogen that causes red crown rot on soybeans (Berner et al., 1991). In-vitro application of glyphosate through growth media inhibited the growth of fungal mycelia. Addition of amino acids to media reversed the effects of glyphosate inhibition, indicating that EPSP synthase inhibition of the fungi causes a depletion of amino acid necessary for growth. Field studies showed a reduction in red crown incidence, agreeing with laboratory studies (Berner et al., 1991). These results indicate that glyphosate can be used in soybeans as both a herbicide as well as a fungicide to control the incidence of *C. crotalariae*.

There are numerous diseases that cause great damage in soybeans. With the release of glufosinate and glyphosate resistant soybean lines, the potential of glufosinate

and glyphosate for controlling bacterial and fungal diseases of soybeans should be investigated. This has the possibility of allowing the producer one pesticide treatment which will control both weeds and disease while leaving the crop undamaged.

1.7 RESEARCH OBJECTIVES

The objectives of this research were to first to conduct whole plant studies investigating the efficacy of glufosinate and glyphosate alone and in combination with additives on weeds and soybeans. Specifically, experiments were conducted to determine if annual and perennial weed species differ in their tolerance to glufosinate and glyphosate applications. Also, research to investigate the possible synergistic effects of the additives ammonium sulfate and pelargonic acid on glufosinate and glyphosate efficacy on weeds, and to determine whether applications of glyphosate or glufosinate in combination with ammonium sulfate or pelargonic acid on Roundup-Ready® and Liberty-Link® soybeans are injurious was conducted.

The second objective was to investigate the mechanism of differential glufosinate sensitivity and the effects of additives on glufosinate efficacy in the five weed species. More specifically, to conduct experiments in order to determine whether absorption, translocation, or metabolism of ^{14}C-glufosinate varies between annual and perennial weed species and whether ammonium sulfate or pelargonic acid affect ^{14}C-glufosinate absorption, translocation, or metabolism in these weeds. And finally, studies to determine whether ammonium sulfate and pelargonic acid affect absorption, translocation, or metabolism of ^{14}C-glufosinate or glyphosate in Liberty-Link® or Roundup-Ready® soybeans were also conducted.

The third objective was to investigate the effects of temperature on the safety of transgenic soybeans to herbicide applications. Specifically, studies were conducted to determine if variable temperatures affect the sensitivity of Liberty-Link® and Roundup-Ready® soybeans to applications of glufosinate or glyphosate, and to determine the basis of this temperature dependent sensitivity by using ^{14}C-glyphosate and glufosinate to monitor absorption, translocation, and metabolism at varying temperatures.

The fourth objective was to determine whether the herbicide glufosinate inhibits the growth of the soybean bacterial pathogen *Pseudomonas syringae* pv. *glycinea* both in-vivo and on inoculated Liberty-Link® soybean trifoliolates.

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