5. EVALUATION OF THE BACTERIOCIDAL ACTIVITY OF THE HERBICIDE GLUFOSINATE ON *PSEUDOMONAS SYRINGAE* PATHOVAR GLYCINEA

5.1 ABSTRACT

Glufosinate, a nonselective, microbially derived herbicide is now being used in Liberty-Link®, glufosinate resistant soybeans. Studies were conducted on *Pseudomonas syringae* pathovar glycinea (L-529) to determine its sensitivity to the herbicide glufosinate. Bacteria were grown in Davis minimal media supplemented by yeast extract containing concentrations of glufosinate between 10 μM and 100 mM. Measurements of turbidity development over time at each different glufosinate concentrations were used to develop growth curves. Bacteria were then inoculated on Liberty-Link® soybeans at the V2 growth stage. Plants were then immediately treated with 0, 0.5, and 1.0 kg/ha glufosinate and kept in a growth chamber at 30°C with a 16-hour day length and 75% relative humidity for 90 hours. Leaf washes were made and bacteria from extract were diluted and plated on *Pseudomonas* Agar F. Bacteria were visually counted to determine whether glufosinate applications inhibited the numbers of live *P. syringae* on soybean leaves. Growth curves showed inhibition of *P. syringae* at glufosinate concentrations greater or equal to 1 mM, and delay of growth at rates as low as 0.01 mM. Liberty-Link® soybeans inoculated with *P. syringae* showed a 45% reduction of live *P. syringae* 3 days after treatment with 0.5 kg/ha glufosinate and a 60% reduction after treatment with 1.0 kg/ha glufosinate.

5.2 INTRODUCTION

Herbicides often inhibit metabolic pathways that are found in both plant and microorganisms. Due to the conservation of many crucial enzymatic binding sites over plants and other organisms, inhibitors of plant enzymes often act as inhibitors of the

respective microbial enzyme. This opens the possibility for herbicides to act as inhibitors of pathogens in some instances.

The herbicide 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 the 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. 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*.

Phosphinothricin, the active ingredient in the herbicide glufosinate, was originally isolated from *Streptomyces* species. Some pathovars of *Streptomyces* produce phosphinothricin naturally as a defense mechanism against other pathogens. When rice transformed with the *pat* gene conferring glufosinate resistance was treated with glufosinate, the herbicide application decreased the symptoms of rice blast disease, caused by the fungus *Magnaporthe grisea* (Tada et al., 1996) and prevented infection by the pathogen *Rhizoctonia solani*, causing sheath blight in rice (Uchimiya et al., 1993).

There are numerous disease-causing pathogens that damage soybeans. With the release of glufosinate and glyphosate resistant soybean lines, the opportunity for glufosinate and glyphosate to control bacterial and fungal diseases of soybeans should be investigated. Lee et al. (1999) found that growth media containing formulated glyphosate (Roundup-Ultra®) reduced significantly the growth of white mold (*Sclerotinia sclerotiorum*).

Application of results from research demonstrating the ability for a herbicide to control microorganisms has the possibility of allowing the producer to apply one pesticide treatment which will control both weeds and disease while leaving the crop undamaged.

5.3 MATERIALS AND METHODS

5.3.1 Growth Curves

The *Pseudomonas syringae* pv. glycinea L-529 from the Lacy bacterial collection at Virginia Tech was cultured on plate count agar. Flasks containing 40-mls of Davis minimal media (Davis and Mingoli, 1950) supplemented with 1 g/L yeast extract were prepared and treated with formulated glufosinate (Liberty® herbicide, 200 g ai/L) or technical grade glufosinate (99% active ingredient) to make concentrations of 0, 0.01, 0.1, 1, 10, 100 mM glufosinate and autoclaved. Flasks were inoculated with 1-ml of *P. syringae* grown overnight in yeast extract supplemented Davis minimal media. Turbidity development was measured over time using a 3-ml aliquot from each flask and measuring turbidity at 600 nm in a spectrophotometer. Replicate flasks with 0.01, 0.1, 1 mM technical grade glufosinate were used as positive controls and the experiment was repeated.

5.3.2 Activity of glufosinate on inoculated soybeans

Liberty-Link® soybeans (var. 5547 LL) were grown in Styrofoam cups containing metro-mix in a growth chamber (30° C, 75% relative humidity, 16-hour day length) until they reached the V2 growth stage. Plants were then inoculated with *Pseudomonas syringae* grown in 40-mls of Davis minimal media supplemented with 1 g/L yeast extract. Bacteria were delivered to plants using a track sprayer applying 237 L/ha output. Immediately following inoculation, plants were treated with 0, 0.5, or 1.0 kg/ha glufosinate and returned to the growth chamber. Trifoliolates were harvested 90 hours after treatment. The center leaflet of the first trifoliolate was collected from 5 plants and washed in 50-ml of water. The wash was centrifuged at 6,000 rpm for 10 minutes to obtain a pellet containing the bacteria. The pellet was re-suspended in 1000-µl water. The bacteria were then diluted using 10-fold dilutions, and replicate plated on *Pseudomonas* Agar F media (Difco Co., Detroit, MI). Counts of colonies were made by

visually counting the number of *P. syringae* colonies on each plate. Three replicates of each treatment were used and the experiment was repeated.

5.4 RESULTS AND DISCUSSION

5.4.1 *Pseudomonas syringae* response to glufosinate

Growth curves showed a glufosinate concentration dependent reduction in growth rate of *Pseudomonas syringae*. Non-treated cultures reached a level of turbidity represented by 0.5 measured at 600 nm wavelength only 14 hours after treatment. Cultures containing 0.01, 0.1, and 1 mM glufosinate reached this level of turbidity only at 18, 23, and 40 hours after treatment respectively. Cultures containing 10 mM glufosinate grew slightly after 27 hours, but those containing 100 mM glufosinate did not grow (Figure 5.1). When comparing treatments containing technical grade glufosinate to those containing the formulated, at 0.01, 0.1, and 10 mM glufosinate the curves for formulated and technical are very similar. However, at 1 mM glufosinate, the technical glufosinate treatment showed significantly more growth than the formulated treatment, suggesting that at this concentration the surfactants contained in the formulated glufosinate may have inhibited growth. At low concentrations of glufosinate, this effect was diluted, and at high glufosinate concentrations, the glufosinate was already toxic to the bacteria so there was no increased toxicity with the addition of the surfactants from the formulation. These growth curves suggest that while low concentrations of glufosinate inhibited the growth of *P. syringae*, after time, the turbidity representing the amount of bacteria was unchanged from that of the non-treated. Higher glufosinate concentrations seemed to either cause bacteria to grow extremely slow, or not grow at all.

This level of inhibition of pathogen growth was similar to that found by Ahmad et al. (1995), who found that a concentration of 1 mM glufosinate reduced the competitive ability of *Trichoderma* species over *Bacillus subtilis.*

5.4.2 Inhibition of *P. syringae* on soybean leaves following glufosinate treatment

The number of live *P. syringae* isolated from Liberty-Link® soybean leaves following treatment with glufosinate was decreased as the rate of glufosinate increased. Leaves treated with 0.5 kg/ha glufosinate showed a 45% decrease in the number of live *P. syringae*, and leaves treated with 1.0 kg/ha glufosinate showed a 60% reduction (Figure 5.2). However, a large proportion of the bacteria inoculated on leaves at the time of treatment died by 3 days after treatment on soybean leaves as determined by an initial leaf wash 1 hour after inoculation. This finding indicates that culture conditions on Liberty-Link® soybeans were not conducive to bacterial growth. Further studies could be conducted using growing conditions that are more conducive to bacterial growth to verify these findings.

In conclusion, glufosinate, an inhibitor of glutamine synthesis and a herbicide, has some growth inhibition activity on *P. syringae* pv. glycinea. Whole plant studies validate that at typical field rates of glufosinate applied to Liberty-Link® soybeans, the number of live *P. syringae* is reduced. However, we did not investigate the effect of glufosinate on actual disease development and these studies would need to be done before recommending glufosinate applications for control of soybean blight. From growth curves, it would appear that glufosinate does have some activity at high concentrations, however, these concentrations are much higher than those typically used for bacterial inhibition by bactericides. This would suggest that present bactericides might be more effective at controlling *P. syringae*. Further studies would need to compare the I_{50} values of glufosinate to those of known bactericidal compounds. Since the actual concentration of an applied herbicide on a leaf surface is very variable, and when the herbicide dries on the leaf surface, the concentration becomes infinite, field doses of glufosinate could be sufficient to control bacteria that come in contact with the herbicide droplet.

Although this study is far from conclusive, it appears that applications of glufosinate to Liberty-Link® soybeans may have some effect on the amount of viable *P. syringae* pv. glycinea remaining on the treated leaf surface. Whether disease outbreak could be controlled by glufosinate applications remains to be determined.

5.5 LITERATURE CITED

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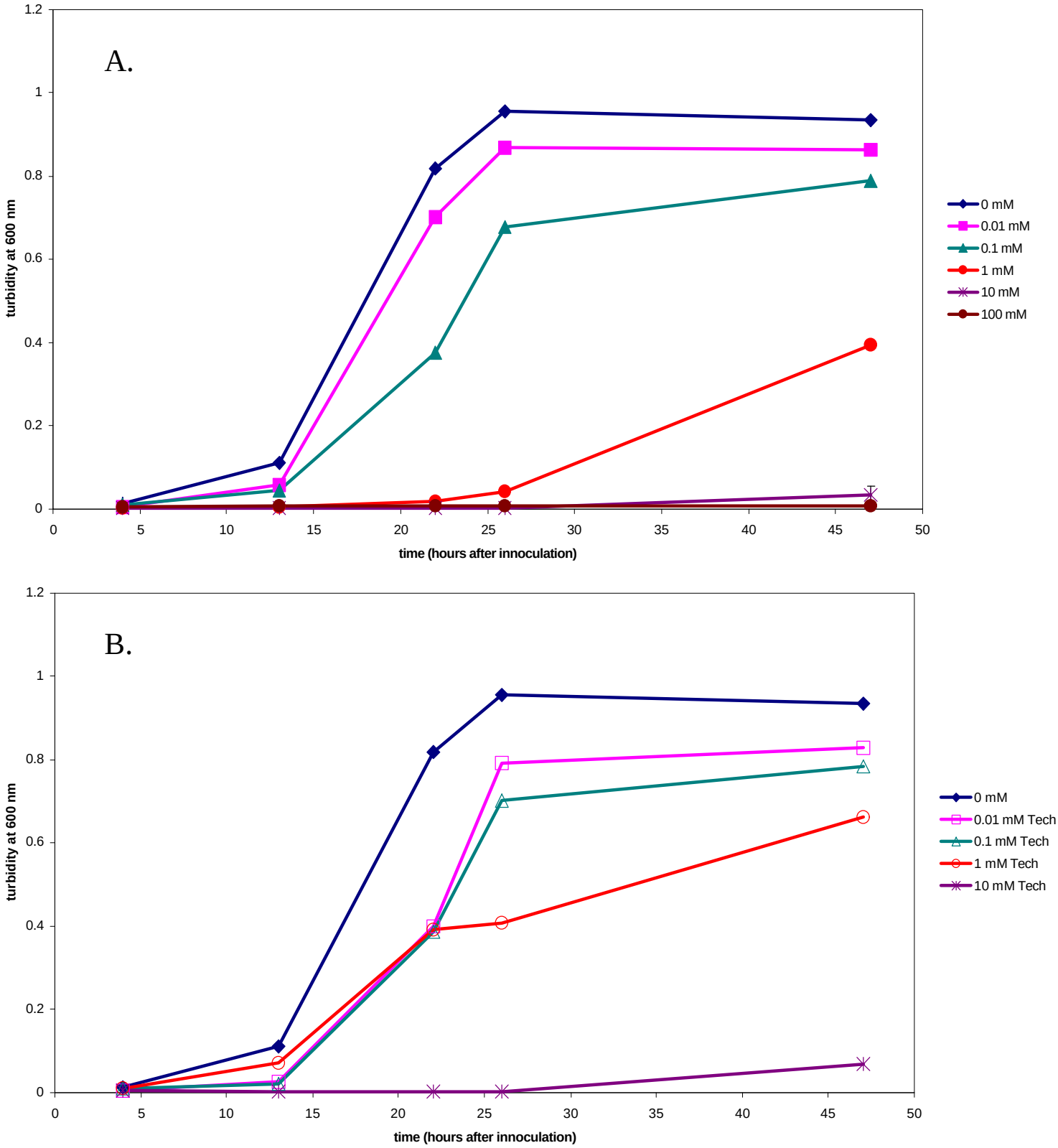

Figure 5.1. Glufosinate inhibition of growth of *Pseudomonas syringae* L-529 as measured over time.

A. Treatments containing formulated glufosinate (Liberty®) at various concentrations. B. Treatments containing technical grade glufosinate (99% purity) at various concentrations. Turbidity development was measured by reading absorbance of cultures at 600 nm wavelength.

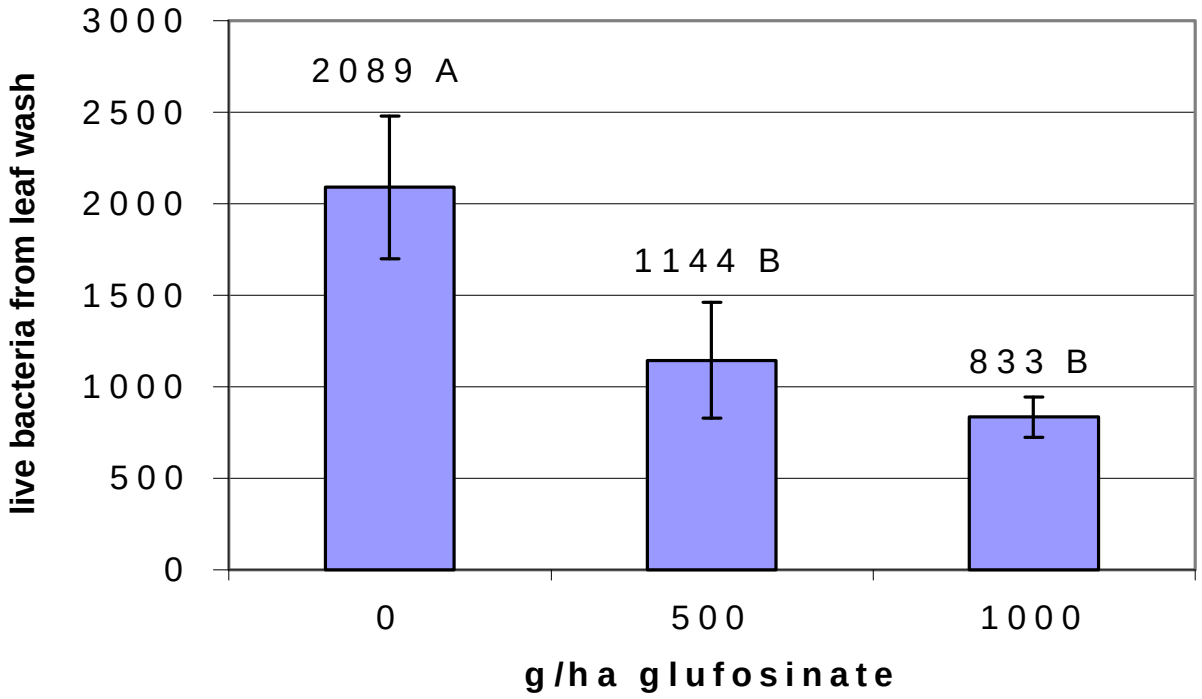

Figure 5.2. Effect of glufosinate treatments on live *Pseudomonas syringae* L-529 isolated from leaf washes of center leaflets of 5 trifoliolates of each treatment.

Leaf washes were made 3 days after inoculation and herbicide treatment. Means were separated using Fishers Protected LSD test at alpha=0.05. Means with the same letter are not significantly different.