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# Effects of Glyphosate and Dalapon on Total Free Amino Acid Profiles of *Pseudosuccinea columella* Snails

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Glyphosate is a broad spectrum herbicide with high water solubility (1.2% at 25°C). It is one of the most important agrochemical discoveries of this century (Grossbard and Atkinson 1985). Glyphosate is relatively nonselective, although very effective on deeply rooted perennial species of grasses, sedges, and broadleaf weeds (Ahrens 1994). Smith and Oehme (1992) indicated that it is relatively non-toxic in certain animal species and has virtually no effects in some aquatic organisms (the 96-hour $LC_{50}$ in rainbow trout (Oncorhynchus *mykiss,* and other fish range from 86-168 mg/L, and a 48 hr $LC_{50}$ of 780 mg/L in Daphnia). However, the 24 hr $LD_{50}$ for *Pseudosuccinea columella* was 98.9 mg/L (Thompson 1989).

Dalapon is a widely used herbicide for control of annual and perennial grasses. It has been used in sugarcane, sugarbeets, corn, potatoes, grapes, and many other crop and noncrop lands (Ahrens 1994). It is an aliphatic acid herbicide with high water solubility (110 g/100 mL at 22°C). The $LD_{50}$ of dalapon in *P. columella* was 98.7 ppm (Thompson 1989). According to Christian *et al.* (1993), sublethal concentrations (0.1-10 mg/L) of Glyphosate and Dalapon caused a dosage dependent increase in protein and an unequal increase in the total aminotransferase activity in *Pseudosuccinea columella* snails.

*Pseudosuccinea columella* serves as an intermediate snail host of *Fasciola hepatica* (sheep liver fluke). In general, the incidence of *Fasciola hepatica* is directly related to the incidence of the susceptible lymnaeid snails, such as *Pseudosuccinea columella.* The continuing rise of liver fluke infections in domestic ruminants such as cattle, sheep, goats and other vertebrates, including humans has attracted much attention (Santiago and Hillyer 1988). The economic implications of this infection are very high and cause millions of dollars in losses each year.

The runoff of these two herbicides (glyphosate and dalapon) into the aquatic ecosystem, and the tendency of aquatic organisms to accumulate these herbicides in their tissues led to increasing investigations (Grossbard and Atkinson 1985). The exposure of non-target organisms such as *P. columella* to these herbicides is enhanced by their water solubility and extensive usage in the environment. These chemicals may directly affect these non-target organisms by causing developmental, morphological, physiological, immunological and biochemical changes. The objective of this study is to determine the effects of sublethal concentrations of glyphosate and dalapon on the free amino acid pool of *P.*

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*columella.* This study will further elucidate and add new dimensions to our knowledge of metabolic changes occurring in *P.columella* snails as a result of chronic exposure to glyphosate and dalapon.

## MATERIALS AND METHODS

Experimental and control snails, *Pseudosuccinea columella,* were raised in the laboratory in 2-gal plastic containers or glass aquarium containing artificial spring water prepared by mixing the following stock chemicals: 0.5 mL ferric chloride (0.25g/L distilled water); 2.5 mL calcium chloride (11.0 g/L distilled water); 2.5 mL magnesium sulfate (10 g/L distilled water); and 1.25 mL phosphate solution (34 g $KH_2P0_4$ in 500 mL of distilled water). The mixtures were then diluted with 1 liter of distilled water MacInnis and Voge (1970). Water hardness was maintained in each aquarium at 80-120 mg/L measured as $CaCO_3$ DO = 6-8 mg/L, pH = 6.5-8.5 and ammonia nitrate levels were maintained at 2 mg/L. Snails were exposed to sublethal concentrations of glyphosate ranging from 1- 10 mg/L (pH 6.8-7.2). All snails were fed endive lettuce leaves *ad libitum* and each aquarium aerated (Christian and Tate 1983; Thompson 1989).

Glyphosate (97%) and dalapon (98.5%) were obtained from Chem Service (West Chester, PA). A 1% stock solution was prepared every month and kept at 25° ± 2°C. The appropriate concentrations were prepared from the stock solution. Each herbicide concentration (0.1, 1, and 10 mg/L) was measured and placed in fleakers (1000 mL). The herbicide concentrations were poured into culture containers and 10 snails were placed into each concentration and maintained. The snails laid eggs and the immature snails developed within the herbicide solutions. Hatched snails were allowed to mature and develop within the herbicide solution.

Sublethal concentrations of glyphosate and dalapon were determined initially by exposing snails to varying concentrations of glyphosate and dalapon to determine toxicity. *Pseudosuccinea columella* snails (4 wk old) raised in sublethal concentrations of glyphosate and dalapon (1- 10 mg/L) were randomly selected from laboratory cultures. Snails raised in artificial spring water served as controls. The snails from each exposure were deshelled and the wet weight obtained. The free amino acids of *P. columella* snails were isolated from the homogenate of 1 gram of snail tissue in 70% EtOH (Graney and Giesy, 1987).

Quantitative analysis of free amino acids were obtained via thin layer chromatography (TLC) employing the following procedure. Samples and amino acid standards were applied to 20 x 20 cm glass plates with silica gel layers. Spots were applied in 1 mL aliquots using a 10 mL pipette and a designated spotting guide with intermittent drying to achieve a spot diameter not exceeding 2 mm. TLC plates were processed in a glass tank supported by a stainless steel frame in 300 mL of solvent. The solvent system used was butanol/acetic acid/water (8:2:2). The plates were sprayed with a 0.5% ninhydrin in acetone for staining. Identification of amino acids present was based on standard amino acids run simultaneously with the use of $R_f$ values and the color reaction from the detection spray. The quantification of the amino acids concentration was determined using the LKB Ultrascan LX Enhanced Laser Densitometer with integrator. Statistical analysis (Analysis of Variance and Duncan's Multiple Range Test) were performed on all experimental data.

## RESULTS AND DISCUSSION

Table 1 shows the $R_f$ values of the five ammo acids identified in *Pseudosuccinea columella* snail tissue by thin layer chromatography. The five amino acids identified were alanine, glycine, glutamic acid, serine, and threonine. Table 2 shows the average concentrations of free amino acids identified in *Pseudosuccinea columella* snails raised in sublethal concentrations of glyphosate. As the concentration of the herbicide (glyphosate) increased, the concentrations of all amino acids increased. These results suggest a concentration dependent effect. Alanine and glycine were found in greater concentrations than the other three amino acids (serine, threonine, and glutamic acid) in *P. columella* snails exposed to glyphosate. The concentrations of alanine and glycine were found to be statistically different from the control ($p< 0.05$), but not significant within the treatment groups. Glutamic acid and threonine were significantly increased at all concentrations tested (0.1, and 10 mg/L) compared to control (shown in Table 2). Serine only showed a significant increase at 10.0 mg/L glyphosate. Table 3 shows the average concentration of free amino acids (alanine, glycine, serine, threonine, and glutamic acid) found in dalapon raised snails. The concentration of alanine and glycine increased significantly in 1.0 and 10.0 mg/L when compared to controls at $p < 0.05$. Snails raised in dalapon did not show a significant increase in their free glutamic acid when exposed at 0.1, 1 and 10 mg/L concentrations. Serine and threonine were significantly increased only at 10 mg/L dalapon when compared to the control. The amount of free amino acids (alanine, glycine, glutamic acid, serine, and threonine) increased as the concentration of dalapon increased, exemplifying a concentration dependent increase. This means the availability or amount of ammo acids present in snail tissues are increased when the level of dalapon is increased in the environment. The quantitative free ammo acid pool in *Pseudosuccina columella* snails was increased more in snails exposed to glyphosate than in snails exposed to dalapon. The differences in amounts of particular free amino acids available indicates the utilization by these snails of free ammo acids in energy producing cycles. The change suggests the presence of specific regulatory mechanisms. For example, alanine plays an important role in cellular osmoregulation (Hamen 1986; Monohar *et al.* 1972). The change may also indicate how the hepatopancreas of the snails are being affected after exposure to these herbicides. Facultative anaerobes obtain considerable substrate-level phosphorylation by coupling glycolysis with amino acid catabolism and alanine is an end product of glycolysis (Graney and Geisy 1987). Surviving environmental stressors is accomplished by these and other types of mechanisms of adaptations.

**Table 1.** $R_f$ x 100 values of the five free amino acids identified in *Pseudosuccinea columella* tissue on silica gel thin layer chromatography

| | $R_f$ x 100 | |
|---|---|---|
| Amino Acids | Standards | Exposed Snail Tissue |
| Alanine | 20 ± 2 | 20 ± 2 |
| Glycine | 18 ± 2 | 18 ± 2 |
| Glutamic Acid | 27 ± 2 | 27 ± 2 |
| Serine | 50 ± 2 | 50 ± 2 |
| Threonine | 54 ± 2 | 54 ± 2 |

Solvent: Butanol/Acetic Acid/Water (8:2:2)

**Table 2.** Average concentration (μg/mL) of free amino acids in *Pseudosuccinea columella* snails raised in sublethal concentrations of glyphosate

| Amino Acids | Control[1] | 0.1 mg/L[1] | 1.0 mg/L[1] | 10.0 mg/L[1] |
|---|---|---|---|---|
| Alanine | 2.03 | 4.53* | 5.49* | 6.82* |
| Glycine | 1.53 | 4.46* | 5.37* | 6.07* |
| Glutamic Acid | 1.19 | 2.97* | 3.68* | 4.02* |
| Serine | 1.02 | 2.01 | 2.39 | 3.64* |
| Threonine | 1.34 | 3.68* | 3.99* | 4.29* |

[1] Values represent mean concentration of five (5 ) analysis.
* means significantly different from control at P< 0.05.

**Table 3.** Average concentration (μg/mL) of free amino acids in *Pseudosuccinea columella* snails raised in sublethal concentrations of Dalapon

| Amino Acids | Control[1] | 0.1 mg/L[1] | 1.0 mg/L[1] | 10.0 mg/L[1] |
|---|---|---|---|---|
| Alanine | 2.03 | 2.74 | 3.82* | 5.94* |
| Glycine | 1.53 | 2.07 | 2.73* | 4.53* |
| Glutamic Acid | 1.19 | 1.49 | 1.85 | 2.22 |
| Serine | 1.02 | 1.28 | 1.47 | 2.54* |
| Threonine | 1.34 | 1.68 | 2.41 | 3.31* |

[1] Values represent mean concentration of five (5) analysis.
* means significantly different from control at P< 0.05.

The presence of each of the herbicides (glyphosate and dalapon) caused an increase in the quantity of free amino acids, which may ultimately be linked to the increase in fascioliasis where these herbicides are used extensively. The presence of sublethal concentrations of glyphosate and dalapon stimulated significant biochemical and physiological responses in *Pseudosuccinea columella* as Thompson (1989) concluded. It is possible that these herbicides may serve as an energy source for these snails. An increase in energy may then contribute to the increased abundance of snails, therefore, increasing the availability of hosts for infection with *Fasciola heputica.* Thompson's study (1989) rendered evidence that exposure to these two herbicides caused an increase in the egg-laying capacity of *P. columella* snails. The extensive use of herbicides in our environment may be a primary factor contributing to the constant rise in fascioliasis.

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