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Chemical Marker for ALS-Inhibitor Herbicides: 2-Aminobutyric Acid Proportional in Sub-Lethal Applications

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A chemical profiling technique for sub-lethal acetolactate synthase (ALS)-inhibitor herbicides (e.g., sulfonylureas, imidazolines, triazolopyrimidine sulfonanilides, and pyrimidyloxy salicylic) was developed using 2-aminobutyric acid, and was found to be directly proportional to application rates in field studies on two varieties of potato plants. An uncomplicated, benign-by-design analytical method for the determination of 2-aminobutyric acid in plant tissue was developed. The method is simple, fast, and automated, entailing a water−trichloroacetic acid extraction followed by precolumn on-line derivatization using *o*-phthalaldehyde (OPA) solution and liquid chromatographic analyses. Use of reagents and chlorinated organic solvents, and generation of waste, are minimized as compared to other ALS-inhibitor herbicide analytical techniques. Recoveries for a series of fortified plant tissues ranged from 82 to 103%. Two 20-day field trials on two potato varieties, Russet Burbank and Shepody, were conducted during the 2000 and 2001 growing seasons. The study demonstrated that the 2-aminobutyric acid method is an excellent, selective chemical marker technique for ALS-inhibitor herbicides for real world plant matrixes.

KEYWORDS: ALS-inhibitor herbicide; 2-aminobutyric acid; potato; chemical marker

INTRODUCTION

Nontargeted effects from herbicides are under increased attention, and injury to nontarget plants many increase with the growing use of postemergence herbicides. Suspected physical drift, volatilization, sprayer contamination, overspray, or carryover of agrochemicals can result in the need for selective, rapid chemical profiling analytical evaluation, whether residue determination is for assessment of potential crop injury or environmental assessment. In the case of nontarget exposures, this can be an especially difficult task (*1*). Although acetolactate synthase (ALS)-inhibitor herbicides, such as sulfonylureas and imidazolines, are commonly used herbicides, the ability to test for these residues in plant matrixes is analytically challenging. This is especially true for drift, contaminated application equipment, and carryover scenarios in which herbicide concentrations are especially low. Many postemergence herbicides (such as ALS-inhibitor herbicides) have high relative activity and can injure plants at low rates when associated with drift, contamination, and overspray. At the same time, environmental conditions will occasionally cause symptoms that may mimic those seen with some herbicides; therefore, diagnostic tools that can identify the true cause of plant injury are important.

Sulfonylurea and imidazolines (examples of ALS-inhibitor) herbicide analyses on real world matrixes (*2*) is challenging (*3*), due in part to several of the positive chemical attributes of these types of herbicides. One characteristic is the potency of sulfonylurea and imidazolines; consequently, application rates of active ingredient (ai)/acre are very low (e.g., tribenuron-methyl recommended rate on cereal grains is ca. 10 g ai/hectare). Thus, levels present in natural matrixes, even at normal application rates, are very low, and sub-lethal levels are even lower. Another challenge of analyzing sulfonylureas is that these compounds are not very amenable to high-performance liquid chromatography or gas chromatography (without mass spectrometry detection), two of the techniques more commonly used in residue laboratories. Another positive attribute of some ALS-inhibitor herbicides is their low persistence. ALS-inhibitor herbicides have a range of environmental persistence (*4*); however, some last for only a few days (*5*) (half-lives) and others last longer, depending on soil conditions. This can make looking for ASL herbicides after the event challenging, especially as compounded by low levels typical of sub-lethal (e.g., carryover) cases.

The sulfonylurea, imidazolinone, triazolopyrimidine sulfonanilides, and pyrimidyloxy salicylic acid herbicides act by inhibiting branched-chain amino acid (*6*) biosynthesis (*7*) at acetolactate synthase (*8*) (ALS, EC 4.1.3.18). Inhibition of acetolactate synthase (ALS) (*9*) also known as acetohydroxyacid synthase (AHAS) [EC 4.1.3.18], leads to depletion of valine, leucine, and isoleucine (*10*), and accumulation of 2-oxobutyrate (2-oxobutanoate) and its transamination product, 2-aminobutyric

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acid (*12*). The accumulation of 2-aminobutyric acid (2-aba) is not simply a consequence of growth inhibition (*12*), an important attribute to developing a chemical marker test that is selective for ALS-inhibiting herbicides. Although hundreds of herbicides exist, currently only about 20 ALS-inhibiting herbicides are on the market.

Predicting the impact of damage from sub-lethal scenarios is difficult because it is difficult to determine the amount of product that contacts the crop. Many drift and carryover scenarios occur early in the growing season, so the plants may recover from the low exposures. However, if the herbicide exposure is high enough that the plant does not resume normal growth quickly, or if environmental conditions cause additional stress, yields will likely be impacted. The impact of drift should be identified soon after the event (*11*), but the impact of drift on yield may not be possible without measuring field yield.

Ideally, a chemical marker for susceptible plants exposed to ALS-inhibitor herbicides (i.e., sulfonylurea, imidazolinone, triazolopyrimidine sulfonanilides, and pyrimidyloxy salicylic acid herbicides) would be specific to only ALS-inhibitor herbicides, develop rapidly in plant tissue (within a few hours), remain in the plant tissue for a period of time (through the first visible signs of damage), would be easier to chemically extract and analyze, and less expensive. The presence of 2-aminobutyric acid has been shown to be specifically selective for plants exposed to lethal ALS-inhibitor herbicides (*12*). However, 2-aba has not been demonstrated as a chemical marker for ALS-inhibiting herbicides in field studies and at sub-lethal applications. The objectives of this study were to develop a robust, simple, and reproducible chemical profiling technique for 2-aba in plant tissue, and to demonstrate that 2-aminobutyric acid is proportional to sub-lethal exposures of ALS-inhibiting herbicides in potato plants.

EXPERIMENTAL PROCEDURES

Reagents. Following are the reagents used, their sources, and their grades (where applicable): sodium acetate trihydrate, Fisher, HPLC grade; triethylamine, Fisher, HPLC reagent grade; tetrahydrofuran, stabilized, Mallinckrodt analytical grade; acetonitrile, Baker Resi-analyzed, J. T. Baker; methanol, Fisher OPTMA; hydrochloric acid, J. T. Baker; *o*-phthaladehyde, OPA Reagent Pickering Co.; OPA diluent, Pickering Co; *N*,*N*-dimethyl-2-mercaptoethylamine-hydrochloride, thiofluor, Pickering Co.; trichloroacetic acid, Fisher; water 18 MΩ*cm, EASYpure UV, Barnstead. The standard used was primary L(+)-2-aminobutyric acid from Acros Organic (98% purity); standards were prepared in water.

Sampling and Preparation. Plant samples were frozen until analysis. Frozen plant tissue samples were homogenized in a Robocoupe using $N_{2(l)}$, and stored frozen in glass jars. A 3-g plant tissue sub-sample and 3 mL of water (18 MΩ*cm) were placed in a centrifuge tube. The sub-samples were placed in an ice bath and further homogenized for 30 to 60 s. The homogenizer was rinsed with water (3 × 1 mL), 0.5 mL of cold 50% trichloroacetic acid was added, and then the samples were placed in an ice bath for 15 min. The volume was adjusted to 10 mL, the sample was centrifuged, and then an aliquot was filtered (0.45-μm disposable) into an HPLC auto-sampler vial.

On-Line Derivatization Instrumental Analysis. HPLC analysis was performed on a Hewlett-Packard 1100; instrument conditions wereas follows: column, Phenonmenex, LUNA C18(2), 3 μm, 150 × 3.0 mm (or equivalent); mobile phase (gradient). Mobile phase "A" was 2.7 g of sodium acetate trihydrate, 180 μL of triethylamine, 3 mL of tetrahyrdofuran, pH adjusted to 7.2, made up to 1 L; mobile phase "B" was 200 mL of 2.7 g of sodium acetate, pH 7.2, 400 mL of acetonitrile, and 400 mL of methanol. The gradient started at 80% A and 20% B; by 20 min the gradient was 80% B; at 21 min the gradient was up to 100% B; at 28 min the gradient was returned to 20% B. The flow rate was 0.5 mL/min.

The on-line (precolumn) derivatization was performed as follows: 10 μL of OPA (*o*-phthalaldehyde) solution (25 mL of OPA deluent, 1 g of thiofluor, and 50 mg of OPA regent) was drawn; using a 500-μL injection loop, 2 μL of sample was drawn, the needle was dipped into the water vial, and 14 uL seat was mixed in at maximum speed 6 times, then injected. Detection was by fluorescence with excitation at 340 nm, and emission at 450 nm.

Field. Field trials were established during the 2000 and 2001 growing seasons in Franklin County in eastern Washington. In both years, the plots were sited in commercial center-pivot-irrigated potato fields with loamy sand soils, 0.63−0.66% organic matter, and cation exchange capacities (CEC) of 5.9−6.8. Season 2000 field plots pH at the 0−12 in soil depth interval was 6.8, and season 2001 plots soil pH at 0−12 in. was 7.9. Buffer zones were provided around each of the treatment and control plots. The first season field trials were established using Russet Burbank potato, and testing three different ALS-inhibiting herbicides at several treatment levels and an untreated control. The ALS-inhibitor herbicides used were rimsulfuron (Matrix), thifensulfuron-methyl and tribenuron-methyl (Harmony Extra), and imazethapyr (Pursuit). The application rates were 2.25 oz formulated product/acre (79 g a.i./hectare) of Matrix (rimsulfuron), 0.07 oz formulated product/acre of Harmony Extra (thifensulfuron-methyl (2.45 g a.i./hectare,) and tribenuron-methyl (1.23 g a.i./hectare)), and 0.8 oz formulated product/acre of Pursuit (imazethapyr (14 g a.i./hectare)). The application rate of Harmony Extra was 20% of the labeled commercial rate for cereal grains, and the application rate of Pursuit (imazethapyr) was 25% of the labeled commercial rate for alfalfa and grains. The Matrix application rate was 225% the labeled commercial rate for potato. The second season sub-lethal applications of Harmony Extra (thifensulfuron-methyl and tribenuron-methyl) were made on Shepody potatoes using the same field design as discussed above. The application rates for Harmony Extra (thifensulfuron-methyl and tribenuron-methyl) were 0.07 oz formulated product/acre (same as year one) and 0.14 oz formulated product/acre (thifensulfuron-methyl 4.9 g a.i./hectare and tribenuron-methyl 1.46 g a.i./hectare). These application rates were 20% and 40%, respectively, of the commercial rate on cereal grains. Herbicide applications were made at the late tuber initiation growth stage in both seasons. A 15−20-cm portion of the terminal growth of the potato plants was harvested in year one at time zero (≈ 3 h), and at 120, 240, 360, and 480 h after application. In year two the plants were harvested at zero (<1 h), 4, 8, 16, 24, 48, 72, 96, 144, and 240 h. The samples were immediately cooled in the field (ice packs) and then frozen within 1 h. The samples were received from the field and stored in the freezer at <−10 °C. In both the 2000 and 2001 seasons, the herbicides were applied at the middle to late tuber initiation stage.

RESULTS AND DISCUSSION

The 2-aminobutyric acid (2-aba) analytical method performed well in the analysis of potato plant tissue samples as demonstrated with the quality control sample results as shown in **Table 1**. Calibration curves consisted of 0.25, 0.5, 1.0, 6.25, and 25 μg/mL standards, r^2 values were ≥0.997. Retention time was ca. 19 min (**Figure 1**), 2-aba is baseline resolved in the complex potato plant matrixes. Total turn-around-time for instrumental analysis was under 30 min. Fortified samples were spiked at the beginning of the plant extraction technique. Validation fortified plant tissue samples (0.50, 1.00, 3.00, 5.00, 10.0, 30.0, and 50.0 μg/g potato of 2-aba) were analyzed in duplicate or quadruplicate ($n = 26$). The recoveries ranged from 82 to 103%, the mean was 89.7% with a standard deviation of 8.8% (**Table 1**). Check standards of 2-aminobutyric acid analyzed periodically were 79 to 105% of true value. Eight sets of duplicates were analyzed, often on different days, and the percent relative difference averaged less than 5% (4.34%; **Table 1**).

The need for an analytical method to supplement direct analysis of specific herbicides as verification of ALS-inhibitor herbicides exposure, is due to the difficulties in analyzing for individual ALS-inhibitor herbicides directly in plant tissue (*5*).

Table 1. Results of Quality Control Samples for 2-Aminobutyric Acid[a]

QC sample type	*n*	average (μg/g) of duplicates	fortified value μg/g	% av. recovery of spike	SD
duplicate potato	2	2.40	NA	NA	0.20
duplicate potato	2	2.30	NA	NA	0.03
duplicate potato	2	1.00	NA	NA	0.04
duplicate potato	2	1.40	NA	NA	0.10
duplicate potato	2	0.86	NA	NA	0.10
duplicate potato	2	12.9	NA	NA	0.10
duplicate potato	2	1.74	NA	NA	0.02
duplicate potato	2	15.4	NA	NA	2.10
fortified potato	4	NA	0.5	85	3.0
fortified potato	4	NA	1.00	82	5.5
fortified potato	2	NA	3.00	102	28
fortified potato	4	NA	5.00	85	6.9
fortified potato	4	NA	10.0	86	3.9
fortified potato	4	NA	30.0	103	15
fortified potato	4	NA	50.0	85	3.7

[a] *n* = number of determinations. The instrument detection limit is estimated to be ca. 0.1 μg/g. NA = not applicable, AV = average, SD = standard deviation.

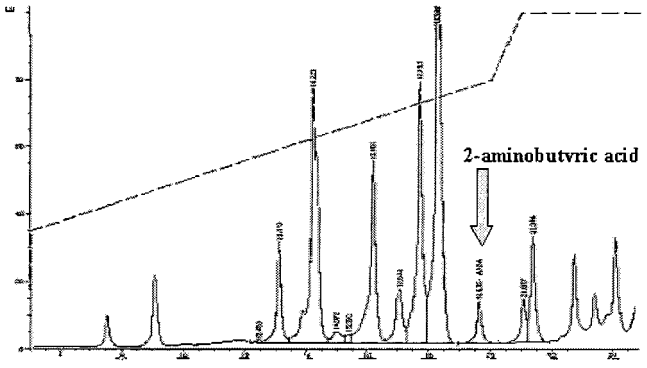

Figure 1. Typical chromatogram of 2-aminobutyric acid in potato tissue.

Most herbicide extractions from plants are labor intensive (*2*, *3*, *5*). For example, a typical extraction for Pursuit (imazethapyr) in potatoes requires a liquid/liquid extraction, solvent exchange, two solid-phase extractions, another solvent exchange, and then analysis by HPLC (*13*). In general, a different method would be required for each type of ALS-inhibiting herbicide; for example, two different methods would typically be used to analyze for sulfonylureas and imidazolines herbicides. Consequently, direct analysis of ALS-inhibitor herbicides in plant matrixes is more expensive, labor intensive, and analytically more difficult than the chemical profiling method we demonstrate here for 2-aminobutyric acid.

Because of the biochemical action of ALS-inhibiting herbicides, specifically the build-up of 2 aminobutyric acid, we intended to take advantage of this exclusive chemical reaction. Rhodes et al. (*12*) has demonstrated that the build-up of 2-aba is not a function of growth inhibition where they investigated several hundred growth inhibitory compounds, but rather, exclusively due to ALS-inhibiting herbicides. Therefore, a chemical profiling technique for the amino acid, 2-aba, was developed.

The extraction technique developed here uses a simple water−trichloroacetic acid extraction. In contrast, other amino acid methods use various hazardous reagents (e.g., picric acid) (*14*) and/or organic solvents while further enrichment is often required prior to analysis. This technique is especially well suited to the analysis of small plant tissue samples, as it requires only 3 g of wet weight tissue, although more tissue may be used. Unlike many amino acid methods (*12*), this technique is not very labor intensive. For example, 12−15 samples can be extracted in less than 3 h. In addition, many amino acid analyses typically require more expensive and/or specialized equipment, such as GC/MS (*15*) or HPLC with a temperature-controlled postcolumn derivatization unit. A more elegant method for common amino acids utilizes on-line (precolumn) derivatization and HPLC analysis (*16*); however, the analysis of 2-aminobutyric acid has never been reported using this technique. This HPLC technique does not require postcolumn derivitization equipment or temperature control equipment. A standard HPLC was used with quaternary pumps; binary pumps were not required. The only nonstandard item used was a 500-μL sample loop. The on-line derivitization reaction was consistent and fast, and all reactions were run at room temperature, another simplifying feature of the technique. The water extraction used here gives excellent recoveries of 2-aba, and provides for clean (interference free) chromatography (**Figure 1)**; 2-aba elutes at 19 min. Detection limits (DL) were determined to be 0.1 μg/g; no further optimization was performed because all samples were already a minimum of a factor of 10 above DL.

An important attribute of using the chemical marker 2-aba versus analyzing directly for ALS-inhibitor herbicides in plant tissues is that 2-aminobutyric acid occurs at relatively high concentrations, ppm versus sub-ppb/ppt, further illustrating its utility as a chemical marker. Also, from an analytical perspective, macro changes are easier to observe (i.e., 1 μg/g normal versus 5−15+ μg/g treated); even at the sub-lethal doses used in this study, looking for a factor of 10 is analytically easier. By screening plant samples for 2-aba, the list of potential causes of plant injury might be clarified. To identify a particular herbicide several analytical methods may need to be performed, for example a capillary electrophoresis method for sulfonylureas, and HPLC for imazethapyr, and so forth. However, the use of a chemical marker method, specific to ALS-inhibiting herbicides, as described here, allows for a single less expensive technique to be used, to test and or screen samples of interest, possibly reducing investigative cost.

Samples from field experiments were analyzed to illustrate a proof-of-concept that a chemical marker may be used for sublethal ALS-inhibitor herbicide applications in two varieties of potato (**Figures 2** and **3**). Control samples in year one were analyzed throughout the study ($n = 9$) and averaged 0.94 ± 0.4 μg/g. In the first season trial, low application rates of Harmony Extra (thifensulfuron-methyl and tribenuron-methyl) and Pursuit (imazethapyr) were applied to Russet Burbank potatoes. Matrix (rimsulfuron), which is a registered ALS-

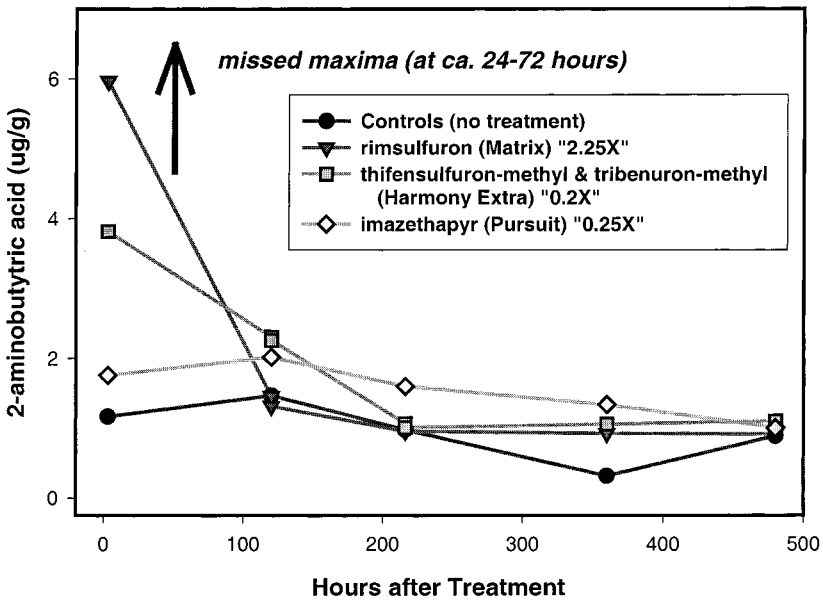

Figure 2. Sub-lethal applications of ALS-inhibitor herbicides on Russet Burbank potatoes. The first samples collected were approximately 3 h after treatments, and 2-aba level was elevated (>50% to 500%) above the control (nontreated). At the next sampling, 5 days later, 2-aba levels decreased and were nearly back to normal; the 2-aba maximum probably occurred between 24 and 48 h after application, see text. The treatment for Matrix is 2.25× the recommended rate for potato; Pursuit and Harmony Extra were applied at 0.25× and 0.2×, respectively, see text.

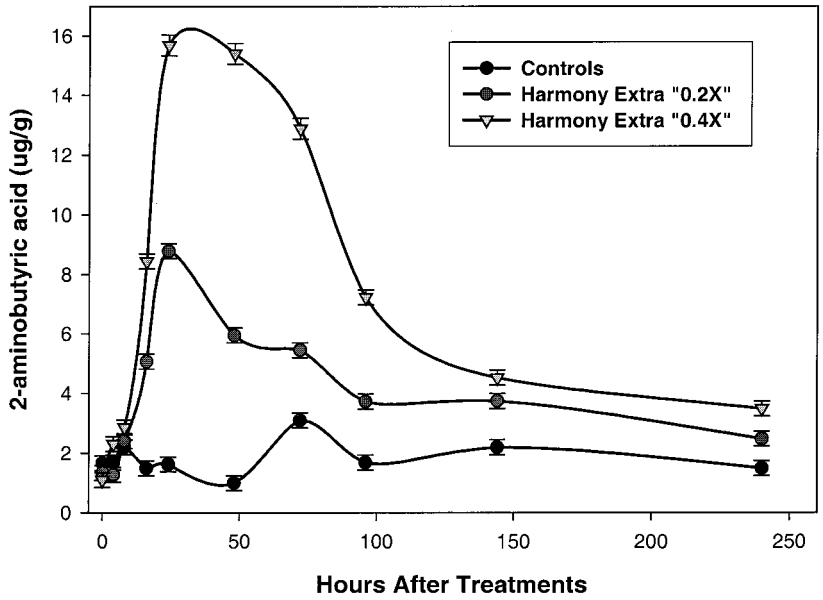

Figure 3. Sub-lethal application(s) of Harmony Extra on Shepody potatoes. Harmony Extra (thifensulfuron-methyl and tribenuron-methyl) 0.2×, application is 20% of the labeled rate, 0.4× is 40% of the labeled rate (see text). Between 4 and 100 hours the 2-aba levels in the 0.4× potatoes are roughly double those in the 0.2× treatment. Error bars for the controls are ±0.5 μg/g ($n = 10$), the other error bars are estimated in part from the duplicates analyzed throughout the study which were <5% relative difference. The error bars are ±0.5 μg/g, or a conservative 10% of the measured value, whichever represented the larger error.

inhibiting herbicide for use on potatoes, was applied at 2.25 × the normal application rate. Russet Burbank potato samples were taken ca. 3 h after treatment. At the postapplication sampling event, 2-aba in Matrix (rimsulfuron)-treated samples was already a factor of 5−6 times higher than that in the controls; 2-aba in Harmony Extra (thifensulfuron-methyl and tribenuron-methyl)-treated samples was about a factor of 3 higher, and in Pursuit (imazethapyr)-treated samples the 2-aba level was 30−50% higher than those in the control samples. Potato plants were sampled again at 5 days (120 h) and the 2-aba levels were returning to normal. The 2-aba maximum was probably missed (additional discussion later). The 2-aba from the Matrix (rimsulfuron) treatment had completely returned to within the range of that of the control plants by 5 days. Even at the high application rate (2.25×) after the first sampling, the Matrix-treated 2-aba levels were statistically the same as those of the untreated samples ($P = 0.4$, paired t-test). Harmony Extra (thifensulfuron-methyl and tribenuron-methyl) also returned to near normal levels by 5 to 10 days and was back to normal at the 20 day sampling. Although Pursuit (imazethapyr) in comparison did not have significant increases of 2-aminobutyric acid levels, these levels remained modestly elevated (ca. 10−50%) until the day 20 sampling when the plants were returning to normal levels. Unlike Matrix, which quickly returned to

normal, the Pursuit (imazethapyr) treatment potatoes were different ($P = 0.0006$, paired t-test) from the control potatoes through 20 days. This may be especially noteworthy because Matrix was applied at 225% and Pursuit was applied at 20% of the recommended application rate: while Matrix did show an initial increase in 2-aba, the Matrix-treated potato quickly returned to normal, but the Pursuit-treated potato (although applied at only 20%) did not return to normal within 20 days. Matrix is registered on potato and Pursuit is not registered for potato. Nonetheless, both ALS-inhibiting herbicides (Matrix and Pursuit) did show increased levels of 2-aba, albeit their response rate and duration are different.

The second season field trials were preformed on Shepody potatoes, and included control and two sub-lethal treatment levels of Harmony Extra (thifensulfuron-methyl and tribenuron-methyl) (20% and 40% of labeled application rates; **Figure 3**). Controls were analyzed throughout the study ($n = 10$) and averaged 1.8 ± 0.6 μg/g. Samples were taken more often early in the study, and significant increases in both treatment levels were seen by 16 h. Results for the low (0.2×) and high (0.4×) treatment samples were a factor of ca. 2 and 4 above the controls, respectively, within only 16 h after treatment. The maximum 2-aba for the 0.2× treatment occurred at ca. 24 h. The maximum 2-aba for the 0.4× treatment was from 24 to 48 h. The 0.2× treatment samples were about a factor of 4 above the control samples at 24 h. The 0.4× treatment samples peaked at about a factor of 8 above the control samples. All treated samples remained substantially elevated through 6 days from the sub-lethal applications and were then modestly elevated at the 10-day sampling.

Both seasonal trials illustrated that 2-aba begins to build up in susceptible plants within a few hours of exposure, so determination of exposure to ALS-inhibitor herbicides can be made prior to symptom expression (**Figures 2** and **3**). An especially interesting result of this study is that we found and quantitated 2-aba in normal plants. Previous studies have indicated that 2-aba represents a zero fraction of the total amino acid pool, as such, the 2-aba may have represented such a small fraction that it was reported as zero, or 2-aba may have been below detection limits with the methods used. In any event, control plants in this study did have small amounts of 2-aba in all plant tissues tested. The controls in year one (Russet Burbank) averaged 0.94 ± 0.42 μg/g 2-aba. The controls in the second season (Shepody potatoes) were 1.82 ± 0.59 μg/g 2-aba. In contrast to some other studies of common amino acids in natural matrixes, our 2-aba levels were remarkable consistent between plants at the same exposure levels (**Table 1**). In an amino acid study of fish (*17*), the authors demonstrated that the levels showed considerable variation; even from the same fish species, a factor of 2 to 4 difference was reported. The authors speculated that this may be due to differences in fish age, sex, and season (*17*). The 2-aba in plants that we measured did not show these variations. This consistency will help when using 2-aba as a diagnostic chemical profiling tool.

The 2-aminobutyric acid is proportional to ALS-inhibitor herbicides exposure and proportional to potato crop injury (**Table 2**). A crop injury rating was performed at the time of field sampling. Crop injury ratings for the control plants, days 0 to 35, were 0 throughout the study period. Crop injury ratings ranged 0−6 from Harmony Extra (thifensulfuron-methyl and tribenuron-methyl) treatments (**Table 2**). Comparing these results to the 2-aminobutyric acid data (**Figures 2** and **3**), 2-aba may be used as a diagnostic character. Yields from treated potatoes were 16 to 21% lower than those from untreated potatoes.

Table 2. Crop Injury on Shepody Potato[a]

hours after treatment	0.2× Harmony Extra[b]		0.4× Harmony Extra[b]	
	foliar	tuber	foliar	tuber
48	0	0	0	0
96	1	0	1.5	0
144	4.5	0.5	5.5	0.5
240	5.0	1	6	1
360	5.5	1	6.5	1
480	3.5	2	4.5	3
672	2.5	3	3.5	4
840[c]	2.0	3	3	4

[a] Crop injury rating scale: 0 to 10, with 0 = no injury and 10=crop death, control foliar and tubers crop injury rating of 0. [b] Harmony Extra is thifensulfuron-methyl and tribenuron-methyl. [c] At harvest, 62 days after application, gross tuber yields were reduced 16–21% in the Harmony Extra (thifensulfuron-methyl and tribenuron-methyl) treatments. Quality of the tubers from the Harmony Extra (thifensulfuron-methyl and tribenuron-methyl) treatments was also significantly degraded.

The method described is simple, fast, precise, and accurate, and it demonstrated good recoveries of 2-aba in potato plant tissues. The extraction technique adequately recovered 2-aminobutyric acid while providing for an interference free chromatogram. The on-line precolumn derivitization is easy, requires no special equipment, is reproducible and robust, and can be done at room temperature. The procedures are fully amenable to programmed operation and autosampling. Turnaround time is reasonably short and analyst interaction time is minimal. Further reduction in turn-around time is probably possible with the use of a 75-mm column, as compared to the 150-mm column used here; a reduction in analysis time down to 10−15 min can be expected (*18*). The method requires minimal sample, which is often a requirement in greenhouse studies. The method minimizes reagents and waste, and greatly reduces hazardous chemicals use compared to that of the currently used methodology. A rough activity-based-cost accounting analysis reveals the cost of a 2-aminobutyric acid plant analysis would be 10 to 25% of similar ALS-inhibitor herbicides plant analyses.

Within this study's experimental parameters, evidence was presented that 2-aminobutyric acid is a good chemical maker for sub-lethal ALS-inhibitor herbicide exposures in potato. At low exposure levels, a period of 3 to 100 hours occurs during which concentrations of 5−8 times the normal 2-aba levels might be expected. After 100 hours, depending on the exposure, the level decline and a smaller increase can be expected.

Other potential applications of this technique may include investigations into ALS-inhibiting herbicide resistance in weeds and selectivity in crops. For instance, common cocklebur (in Mississippi) resistant to imidazolinone herbicides imazaquin (Scepter) and imazethapyr (Pursuit (imazethapyr)) and ryegrass resistant to sulfonylurea herbicide sulfometuron (Oust) have been reported. Alternatively, the development of resistant crops allowing for further herbicide reduction is under development. For example, resistant sugarbeet may increase the limited chemical weed control options by allowing the use of low rates of environmentally friendly, ALS-inhibiting herbicides directly in the sugarbeet crop (*19*). The accumulation of 2-aba may also be a useful method to determine the mechanism of selectivity of ALS-inhibiting herbicides. For example, Matrix (rimsulfuron) is registered for use on potatoes; however, following application at 2.25X the normal recommended rate, it initially elicited a

very rapid and substantial accumulation of 2-aba, which then rapidly dissipated. Whereas in the same field trial, a relatively lower application of the nonpotato registered herbicide Harmony Extra (thifensulfuron-methyl and tribenuron-methyl), resulted in a lesser initial accumulation of 2-aba, but a slower dissipation of the 2-aba concentration in the potato tissue. The technique presented here for 2-aminobutyric acid is a method that could provide a quick reliable way to screen if a specific weed had developed resistance to this class of herbicides, or whether a crop was tolerant to members of this class of herbicides when coupled with control samples. If a weed had developed resistance although 2-aba may initially increase, like the potato Matrix 2.25X treatment, the 2-aba would rapidly return to normal. Whereas for weeds susceptible to the ALS-inhibiting herbicide, 2-aba would remain elevated for days. Comparison of the two 0.2X treatments with Harmony Extra in year one on Russet Burbank and year two on Shepody, are remarkable similar when the 2- aba data is normalized (i.e. 2-aba/average 2-aba untreated). Although the data represents two season on two varieties, and the sampling times were slight different, the difference between 2-aba during the study is less than 0.5ug 2-aba/g. Overall, when used with control samples the 2-aba chemical profiling technique appears to be valid diagnostic tool for ALS-inhibiting herbicides.

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