Influence of Glyphosate on Amino Acid Composition of Egyptian Broomrape [*Orobanche aegyptiaca* (Pers.)] and Selected Hosts

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The parasitic plant broomrape is entirely dependent on its host for reduced carbon and nitrogen and is also susceptible to inhibition by glyphosate that is translocated to the parasite through a host. Studies were conducted to examine the effect of broomrape parasitism on amino acid concentrations of two hosts: common vetch that is tolerant of low levels of glyphosate and oilseed rape that has been genetically engineered for glyphosate resistance. The influence of glyphosate on the amino acid content of broomrape and the two hosts was also examined. Amino acid concentrations in leaves and roots of parasitized common vetch plants were generally similar to those of the corresponding tissues of nonparasitized plants. Amino acid concentrations in broomrape were lower than those of the parasitized common vetch root. For common vetch, glyphosate applied at rates that selectively inhibited broomrape growth did not alter individual amino acid concentrations in the leaves, but generally increased amino acid levels at 0.18 kg ha^{-1}. Glyphosate application also increased the amino acid concentrations, with the exception of arginine, of broomrape growing on common vetch and did not generally influence concentrations in leaves or roots of common vetch. In oilseed rape, parasitization by broomrape generally led to higher amino acid concentrations in leaves but lower concentrations in roots of parasitized plants. Broomrape had higher amino acid concentrations than roots of the parasitized oilseed rape. Glyphosate applied at 0.25 and 0.5 kg ha^{-1} generally increased the amino acid concentrations in oilseed rape leaves, but the 0.75 kg ha^{-1} application caused the amino acid concentrations to decrease compared to those of untreated plants. In oilseed rape root the general trend was an increase in the concentration of amino acids at the two highest rates of glyphosate. Individual amino acid concentrations in broomrape attachments growing on oilseed rape were generally increased following glyphosate application of 0.25 kg ha^{-1}. These results indicate that low rates of glyphosate alter amino acid profiles in both host and broomrape and raise questions about the regulation of amino acid metabolism in the parasite.

Keywords: *Glyphosate; amino acid composition; Egyptian broomrape; Orobanche aegyptiaca Pers.; common vetch; Vicia sativa L.; oilseed rape; Brassica napus L.*

INTRODUCTION

Egyptian broomrape (*Orobanche aegyptiaca* Pers.) is a parasite that attacks the roots of many dicotyledonous crops. It draws carbon, nutrients, and water through haustoria, which connect the parasite with the host's vascular system. Broomrape infestations cause reductions in crop yield, adversely affect crop quality, and result in loss of cultivated land due to reduced crop alternatives (*1*). Commonly affected host crops include legumes such as common vetch (*Vicia sativa* L.) and broad bean (*Vicia faba* L.), cruciferous crops such as oilseed rape (*Brassica napus* L.), and several members of the families Apiaceae, Asteraceae, and Solanaceae. Control of broomrape is difficult because of its close association with the host throughout its life cycle.

Glyphosate is a systemic, nonselective, and foliar-applied herbicide that is readily translocated to underground plant parts, immature leaves, and meristems. Glyphosate acts by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase (EC 2.5.1.19) that catalyzes the production of the aromatic amino acids phenylalanine (Phe), tyrosine (Tyr), and tryptophan (Trp). Since discovery of its herbicidal properties (*2*) and commercial introduction in 1974, glyphosate has been used extensively in both cultivated and noncrop lands. It is generally applied as a preplant or after harvest treatment for broad-spectrum weed control.

Despite its general nonselectivity, a small margin of selectivity at low rates has been reported in certain crops, some of which are broomrape hosts [reviewed by Foy et al. (*3*) and Parker and Riches (*1*)]. Kasasian (*4*) first reported the selective control of crenate broomrape (*Orobanche crenata* Forsk.) with glyphosate applied to broad bean foliage at rates of 0.2−0.3 kg ha^{-1} 6 weeks after sowing. After application to host foliage, glyphosate has been shown to translocate through the host phloem to broomrape attachments on the host roots (*5*).

Although glyphosate has provided good control of broomrape in certain crop situations, the margin of crop safety is very narrow, and applicators' error or environmental factors can lead to either crop injury or lack

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of acceptable broomrape control. The problem of herbicide toxicity to the host plant can be avoided in crops that are genetically engineered for glyphosate resistance. The possibility of herbicide-resistant crops for broomrape control was first addressed by Foy et al. (*3*) and described in detail by Gressel et al. (*6*). Joel (*7*) reported the first utilization of herbicide-resistant crops for broomrape control, demonstrating complete suppression of the parasite by application of chlorsulfuron to parasitized transgenic chlorsulfuron-resistant tobacco (*Nicotiana tabacum* L.).

Recently, four examples of genetically engineered resistance, three target-site and one metabolic, were tested for use in controlling broomrape (*8*). Excellent control of broomrape was obtained with chlorsulfuron in a transgenic tobacco line containing a modified acetolactate synthase (EC 4.1.3.18) enzyme resistant to chlorsulfuron, with glyphosate in oilseed rape plants containing a modified EPSP synthase resistant to glyphosate, and with asulam in tobacco plants having a modified dihydropteroate synthase (EC 2.5.1.15) resistant to asulam. A variety of tomato (*Lycopersicon esculentum* Mill.) engineered for resistance to glufosinate, an inhibitor of glutamine synthetase (EC 6.3.1.2) (GS), was parasitized by broomrape despite application of glufosinate. This parasitism was expected because the transgenic line of tomato metabolized the herbicide to a nontoxic form. In each of the four cases, control plants were severely infested with broomrape. These observations suggest that genetically engineered herbicide-resistant crops have great potential for use as alternative crops in broomrape-infested areas.

It is interesting to examine the effect of glyphosate on the host−broomrape interaction for two reasons. First, the role of broomrape amino acid synthesis can be studied by selectively inhibiting EPSP synthase of broomrape with glyphosate [host's (common vetch or glyphosate-resistant oilseed rape) EPSP synthase should be tolerant to glyphosate]. Second, the impact of sublethal rates of glyphosate on the amino acid composition of these hosts can be assessed. Broomrape has two potential sources of amino acids. First, the haustorium of broomrape serves as a conduit for translocation of amino acids from the host plant to the parasite. Second, broomrape may synthesize some amino acids by itself. However, the relative importance of these two modes of acquiring amino acids by broomrape is not known.

Broomrapes have been found to either lack or possess low activity of nitrogen assimilating enzymes. Lee and Stewart (*9*) were unable to detect nitrate reductase (EC 1.6.6.2) activity in broomrape, which indicates that the parasite is unable to utilize nitrate. Low activity of GS, the enzyme that catalyzes the incorporation of ammonium into organic compounds, was found in broomrape and another holoparasite, *Lathraea clandestina* L. (*10*−*13*). Nitrate reduction in *L. clandestina* has been shown to be incomplete or to proceed at very low rates (*14*−*16*).

The absence or low activity of nitrogen-assimilating enzymes in broomrape may indicate that the parasite relies on organic nitrogen from the host plant. Translocation of amino acids and amides has been shown for broomrape (*17*) and other angiospermous parasites (*10*, *18*−*20*). Broomrape seeds were able to absorb and metabolize radioactive leucine (Leu) during both conditioning and germination, and a part of the Leu was metabolized (*21*).

The objectives of this research were to examine the effect of glyphosate on amino acid levels in broomrape and its hosts, common vetch and oilseed rape, hereafter referred to as vetch and rape, respectively, and to better understand the impact of broomrape parasitism on amino acid composition of the two host crops.

MATERIALS AND METHODS

Plant Material. Seeds of vetch, variety Yovel (Dr. Y. Kleifeld, Newe-Ya'ar Research Center, Israel), and glyphosate-resistant rape (Monsanto Canada, Inc., Edmonton, AB) were surface sterilized in a 1% solution of sodium hypochlorite. Four seeds were planted 2.5 cm below the soil surface in 50-mL plastic centrifuge tubes (11 cm × 2.5 cm) containing a vermiculite/topsoil mixture (3:1 by volume) infested with Egyptian broomrape seeds (Dr. Kleifeld). Both common vetch and oilseed rape plants emerged within 7−10 days of planting, and 2-week-old seedlings were thinned to two per tube. Plants were watered on alternate days and fertilized with half-strength Hoagland's nutrient solution (*22*) once a week. Throughout the experiment, tubes were placed in a growth room with light intensity of 300 μeinstein m^{-2} s^{-1} provided by fluorescent lights, and the temperature was 24 °C during the 12-h light period and 20 °C during the night.

Glyphosate Dose Response. A commercial formulation of glyphosate (Monsanto Co., St. Louis, MO) was applied at 0.09, 0.18, and 0.36 kg of acid equivalent (ae) ha^{-1} to vetch, and 0.25, 0.5, and 0.75 kg of ae ha^{-1} to oilseed rape. In a preliminary experiment, rape plants were treated with glyphosate at 0.75 kg ha^{-1} to confirm resistance to glyphosate. A nonionic surfactant (X-77, Valent USA Corp., Walnut Creek, CA) was added at 0.25% (v/v) to all glyphosate treatments. All of the herbicide treatments were applied 5 weeks after planting with a greenhouse sprayer equipped with a Teejet 8001E nozzle tip (Spraying Systems Co., Wheaton, IL) delivering a volume of 200 L ha^{-1} at 220 kPa. One week after treatment, fresh weights were taken of host shoot and root and broomrape attachments, and the number of live broomrape attachments was counted on both common vetch and oilseed rape. Untreated parasitized plants of vetch and rape were used as the respective controls.

Immediately after harvest, all tissues were frozen in liquid nitrogen and lyophilized. Dried tissues, except dry and germinated seed, collected over several months and stored at −20 °C were pooled, ground to pass a 1-mm screen using a Udy (Ft. Collins, CO) cyclone mill, and stored at −20 °C until analyzed. Subsamples were used for chemical analyses, and results are expressed on an "as is" dry weight basis.

Preparation of Samples for Amino Acid Analysis. For amino acid determinations, duplicate subsamples of 0.02 g of each tissue were weighed into 5-mL glass ampules. After the addition of 3 mL of 6 N HCl, ampules were immediately purged with grade 5 nitrogen and sealed, and samples were hydrolyzed for 6 h in a Thermatic 60 (Castle Sybron, Rochester, NY) autoclave set at 132 °C. Hydrolysates were transferred to 5-mL volumetric flasks, 1 mL of internal standard solution (α-aminobutyric acid in 0.1 N HCl, 5 μmol/mL) was added, and the volume was brought to 5 mL with MilliQ water (Millipore, Bedford, MA). Aliquots filtered through a 0.45-μm Teflon syringe filter were used for the amino acid derivatization sequence. Tryptophan and the S-containing amino acids, cysteine and methionine, which are partly or completely destroyed by acid hydrolysis, were not analyzed.

Chemical Analyses. Amino acids were analyzed as phenylthiocarbamyl derivatives using a modification of the procedure of Bidlingmeyer et al. (*23*). The high performance liquid chromatographic (HPLC) system consisted of a Perkin-Elmer (Norwalk, CT) series 200 quaternary gradient liquid chromatographic pump, a Perkin-Elmer diode array model 235 C detector (254 nm), a Perkin-Elmer series 200 autosampler equipped with a peltier accessory, and a Waters Pico-Tag free amino acid analysis column (3.9 mm × 30 cm). Column temperature was maintained at 36 °C with a Perkin-Elmer series 200 column oven. Eluents were laboratory-prepared and

Table 1. Gradient Table for Analysis of Phenylthiocarbamyl Derivatives of Amino Acids Using a Perkin-Elmer Series 200 Quarternary Pump[a]

step	time	flow	%A[b]	%B[c]	curve
0	5.0	1.0	100	0	0
1	1.0	1.0	100	0	0
2	5.0	0.6	95	5	1
3	15.5	1.0	54	46	1
4	7.5	0.6	0	100	1
5	2.0	1.2	0	100	0
6	6.0	1.4	0	100	0
7	2.0	1.4	100	0	1
8	2.0	1.4	100	0	0
9	2.0	1.2	100	0	0

[a] Detector: Perkin-Elmer diode array model 235 C (254 nm). Column oven: Perkin-Elmer series 200 (36 °C). Autosampler: Perkin-Elmer series 200 equipped with peltier accessory. Pump: Perkin-Elmer series 200 quarternary gradient liquid chromatography pump. Data System: Perkin-Elmer-Nelson Turbochrome 4. [b] Eluent A: 0.14 M sodium acetate, 0.05% (v/v) triethylamine, pH 6.40 containing 6% (v/v) acetonitrile. [c] Eluent B: 60% (v/v) acetonitrile in water.

sparged with helium using a Perkin-Elmer solvent module. Separation of sample components was accomplished using the gradient listed in Table 1. Data acquisition and peak quantification were accomplished using a PE Nelson (Norwalk, CT) Turbochrome 4 chromatographic data system. Protein amino acid standard solution (standard H) and PITC were purchased from Pierce Chemical Co. (Rockford, IL). Homoserine and individual protein amino acids were obtained from Sigma Chemical Co. (St. Louis, MO). Other chemicals were of the highest purity available.

Safety. The amino acid derivatizing reagent, PITC, reacts on contact with strong acids, emitting highly toxic cyanide fumes and/or oxides of sulfur.

RESULTS AND DISCUSSION

Amino Acid Composition of Vetch and Broomrape. To understand the effect of parasitism on host plants and how the host influences the amino acid composition of the parasite, we compared parasitized and nonparasitized host plants and their associated parasites (Table 2). Amino acid concentrations in leaves and roots of parasitized vetch plants were generally similar to those of the corresponding tissues of nonparasitized plants. Amino acid profiles from broomrape, on the other hand, showed some striking differences as compared to host roots. The greatest of these is the elevated level of Arg in the parasite compared to that of the parasitized root. Because Arg is high in nitrogen, it may be that broomrape preferentially accumulates high-nitrogen amino acids. Levels of all the other amino acids in broomrape were lower than those of the parasitized root.

Amino Acid Composition of Glyphosate-Treated Vetch and Broomrape. The amino acid composition of hydrolysates of glyphosate-treated tissues of vetch and associated broomrape is presented in Table 2. Glyphosate treatments did not cause any change in individual amino acid concentrations in the leaves of vetch. On the other hand, as the rate of glyphosate increased from 0 to 0.18 kg ha^{-1} there was a trend toward increased amino acid concentrations in roots of vetch. This lack of inhibitory effect of glyphosate on amino acid concentrations could be related to the tolerance of certain legumes, such as vetch (*24*) and broadbean (*4*), to low rates of glyphosate. However, levels of most amino acids declined toward untreated levels at the 0.36 kg ha^{-1} rate. Almost 20% of the carbon fixed by photosynthetic plants flows through the shikimate pathway (*25*). Application of glyphosate at a high rate of 36 kg ha^{-1} may have changed the activity of certain enzymes in the vetch plants, which has impaired the supply of carbon skeletons for synthesis of amino acids needed for amino acid-synthesizing enzymes as well as enzymes of other metabolic pathways. With respect to broomrape attachments growing on vetch, all rates of glyphosate dramatically increased the amino acid concentrations, with the exception of arginine (Arg).

Amino Acid Composition of Rape and Broomrape. In rape, leaves of parasitized plants had generally higher concentrations of amino acids than those of nonparasitized plants, with the exception of Pro (Table 3). Conversely, roots of parasitized rape had lower amino acid concentrations than those of nonparasitized plants. Unlike vetch described above, broomrape parasitizing rape had generally higher amino acid concentrations than the host roots. As seen with broomrape on vetch, broomrape on rape accumulated Arg at remarkably high levels.

Amino Acid Composition of Glyphosate-Treated Rape and Broomrape. Amino acid concentrations of untreated and glyphosate-treated tissues of rape and associated broomrape attachments are presented in Table 3. Glyphosate treatments of 0.25 and 0.5 kg ha^{-1} generally resulted in increased concentrations of amino acids in rape leaves. The aromatic amino acids, Phe and Tyr, did not differ from this pattern. This is not surprising because it indicates glyphosate did not seem to impact the shikimate pathway of rape, which is glyphosate-resistant. Cooley and Foy (*26*) have reported increased levels of free amino acid pools in inflated duckweed (*Lemma gibba* L.) after glyphosate treatment. Analysis of free amino acids would further explain the increase in amino acid levels of the host and the parasite. Glyphosate application at 0.75 kg ha^{-1} caused the amino acid concentrations of rape leaves to fall below those of leaves of untreated plants. The amino acid concentrations of the roots of plants treated with glyphosate at 0.25 kg ha^{-1} were similar to those of roots of untreated plants. Glyphosate applications of 0.5 and 0.75 kg ha^{-1} increased concentrations of individual amino acids of rape roots. The general increase in amino acid concentrations of the leaves and roots of rape in response to glyphosate may be a response to stress, as increased protein turnover rates are known to occur under stress conditions. This indicates that, despite having a target-site resistance for glyphosate, the rape plants suffer alterations in metabolism following glyphosate treatments. Amino acid concentrations in broomrape growing on rape were either unchanged or elevated when glyphosate was applied at 0.25 kg ha^{-1}. Only tubercles from the lowest glyphosate rate were included because the higher rates effectively prevented tubercle growth. It is noteworthy that this pattern was consistent for Tyr and Phe. This increase in aromatic amino acid concentrations in broomrape is an interesting development because broomrape growth was significantly suppressed by glyphosate (*24*). Glyphosate kills plants through the inhibition of EPSP synthase, which catalyzes a step in the aromatic amino acid biosynthetic pathway. It is likely that broomrape has functional EPSP synthase, as evidenced by its susceptibility to glyphosate, but direct research on this enzyme is lacking in parasitic plants. Although plastids of holoparasites such as broomrape are not photosynthetically active,

Table 2. Amino Acid Composition of Nonparasitized and Glyphosate-Treated Broomrape-Parasitized Tissues of Common Vetch and Associated Broomrape Attachments[a]

	concentration, μmol/g of DW[b]												
	common vetch										broomrape attachment			
	leaf					root								
amino acid	nonparasitized	parasitized, glyphosate, kg of ae/ha				nonparasitized	parasitized, glyphosate, kg of ae/ha				glyphosate, kg of ae/ha			
		0	0.09	0.18	0.36		0	0.09	0.18	0.36	0	0.09	0.18	0.36[c]
Asx[d]	76 ± 6	80 ± 3	86 ± 4	82 ± 9	84 ± 15	87 ± 4	97 ± 5	103 ± 5	117 ± 2	109 ± 3	72 ± 19	96 ± 8	102 ± 5	98
Glx[d]	80 ± 9	80 ± 2	81 ± 3	80 ± 2	81 ± 7	85 ± 5	85 ± 3	88 ± 2	104 ± 1	95 ± 1	74 ± 18	103 ± 7	113 ± 4	111
Ser	46 ± 5	44 ± 2	47 ± 1	45 ± 2	48 ± 1	74 ± 6	80 ± 4	81 ± 2	94 ± 1	83 ± 1	48 ± 11	67 ± 3	70 ± 4	69
Gly	96 ± 10	97 ± 0	100 ± 1	98 ± 0	101 ± 3	91 ± 4	92 ± 1	95 ± 0	110 ± 2	100 ± 0	77 ± 20	110 ± 3	119 ± 1	120
His	12 ± 1	12 ± 2	12 ± 2	11 ± 2	15 ± 0	23 ± 1	24 ± 1	25 ± 0	30 ± 0	26 ± 0	14 ± 3	19 ± 2	19 ± 1	18
Arg	48 ± 5	48 ± 0	47 ± 2	47 ± 1	51 ± 4	38 ± 2	39 ± 1	41 ± 1	44 ± 0	40 ± 1	52 ± 13	57 ± 4	54 ± 2	52
Thr	44 ± 3	41 ± 6	43 ± 0	43 ± 1	49 ± 2	56 ± 8	61 ± 1	63 ± 2	72 ± 0	62 ± 5	42 ± 10	60 ± 0	61 ± 5	63
Ala	71 ± 7	68 ± 4	69 ± 1	69 ± 3	72 ± 2	93 ± 5	94 ± 2	97 ± 1	109 ± 1	100 ± 0	70 ± 17	99 ± 4	105 ± 2	109
Pro	53 ± 5	54 ± 2	54 ± 0	53 ± 0	56 ± 2	61 ± 2	66 ± 1	66 ± 1	76 ± 1	71 ± 0	43 ± 9	60 ± 1	60 ± 1	61
Tyr	21 ± 2	21 ± 1	20 ± 1	20 ± 1	22 ± 1	26 ± 2	26 ± 0	28 ± 2	31 ± 2	27 ± 0	17 ± 5	26 ± 2	27 ± 1	25
Val	49 ± 6	46 ± 4	47 ± 2	46 ± 4	49 ± 1	84 ± 4	85 ± 2	87 ± 1	99 ± 2	90 ± 0	58 ± 14	82 ± 3	84 ± 2	84
Ile	36 ± 5	34 ± 3	35 ± 1	33 ± 3	36 ± 0	58 ± 3	57 ± 1	58 ± 0	66 ± 1	60 ± 1	43 ± 10	61 ± 3	61 ± 1	60
Leu	70 ± 9	67 ± 2	69 ± 2	65 ± 4	70 ± 1	87 ± 4	84 ± 2	86 ± 1	100 ± 2	91 ± 0	69 ± 17	97 ± 4	97 ± 2	95
Phe	36 ± 5	36 ± 1	37 ± 0	36 ± 0	37 ± 2	43 ± 2	43 ± 1	44 ± 1	51 ± 1	46 ± 0	31 ± 8	44 ± 1	45 ± 1	44
Lys	42 ± 4	41 ± 2	42 ± 0	38 ± 0	39 ± 2	71 ± 2	69 ± 4	74 ± 2	84 ± 3	76 ± 1	46 ± 11	64 ± 3	61 ± 2	54

[a] Amino acids in tissue hydrolysate are listed according to the elution sequence of phenylthiocarbamyl derivatives separated by HPLC on a Waters Pico-Tag free amino acid column (3.9 × 3.0 cm). DW, dry weight. [b] Mean values and standard deviations are for pooled tissue samples; duplicate analyses were performed for each tissue. [c] Single analysis was performed. [d] Asx and Glx represent asparagine + aspartate and glutamine + glutamate, respectively.

Table 3. Amino Acid Composition of Nonparasitized and Glyphosate-Treated Broomrape-Parasitized Tissues of Oilseed Rape and Associated Broomrape Attachments[a]

	concentration, μmol/g of DW[b]													
	oilseed rape										broomrape attachment			
	leaf					root								
amino acid	nonparasitized	parasitized, glyphosate, kg of ae/ha				nonparasitized	parasitized, glyphosate, kg of ae/ha				glyphosate, kg of ae/ha			
		0	0.25	0.50	0.75		0	0.25	0.50	0.75	0	0.25	0.50[c]	0.75[c]
Asx[d]	50 ± 4	64 ± 24	71 ± 2	75 ± 1	46 ± 14	33 ± 3	26 ± 2	25 ± 2	29 ± 2	32 ± 6	56 ± 2	64 ± 3		
Glx[d]	41 ± 4	55 ± 27	63 ± 7	66 ± 3	37 ± 11	53 ± 6	41 ± 0	43 ± 1	59 ± 1	56 ± 12	61 ± 2	68 ± 1		
Ser	27 ± 0	33 ± 12	39 ± 1	43 ± 3	25 ± 8	45 ± 3	37 ± 1	36 ± 2	46 ± 1	44 ± 6	45 ± 2	46 ± 1		
Gly	92 ± 6	97 ± 20	98 ± 7	103 ± 3	75 ± 11	62 ± 3	52 ± 3	47 ± 0	59 ± 0	57 ± 7	71 ± 5	78 ± 1		
His	5 ± 1	8 ± 4	10 ± 1	9 ± 1	5 ± 1	13 ± 0	10 ± 1	11 ± 0	14 ± 2	14 ± 2	13 ± 0	15 ± 1		
Arg	42 ± 1	48 ± 9	49 ± 2	52 ± 0	39 ± 6	35 ± 2	28 ± 1	30 ± 0	34 ± 1	33 ± 4	80 ± 4	114 ± 0		
Thr	21 ± 2	29 ± 6	33 ± 2	37 ± 6	17 ± 7	38 ± 1	31 ± 0	29 ± 1	36 ± 1	35 ± 5	39 ± 2	39 ± 3		
Ala	30 ± 5	44 ± 24	62 ± 1	62 ± 7	22 ± 8	60 ± 3	50 ± 1	46 ± 1	57 ± 0	53 ± 8	68 ± 3	71 ± 1		
Pro	70 ± 2	67 ± 7	66 ± 2	72 ± 2	57 ± 6	39 ± 2	32 ± 2	31 ± 2	38 ± 1	36 ± 4	40 ± 5	38 ± 0		
Tyr	8 ± 1	9 ± 4	11 ± 0	12 ± 0	7 ± 2	18 ± 1	13 ± 0	14 ± 0	18 ± 0	18 ± 2	19 ± 1	22 ± 1		
Val	8 ± 5	18 ± 16	32 ± 4	29 ± 5	8 ± 3	49 ± 2	41 ± 2	38 ± 0	47 ± 2	46 ± 6	50 ± 1	52 ± 1		
Ile	6 ± 4	10 ± 9	22 ± 2	20 ± 4	5 ± 1	31 ± 1	25 ± 1	22 ± 0	28 ± 1	27 ± 4	36 ± 1	38 ± 1		
Leu	20 ± 8	35 ± 25	55 ± 4	52 ± 8	16 ± 5	54 ± 3	42 ± 3	38 ± 1	48 ± 2	47 ± 7	59 ± 1	62 ± 0		
Phe	25 ± 3	32 ± 14	37 ± 2	38 ± 3	19 ± 5	25 ± 1	20 ± 1	19 ± 0	24 ± 1	23 ± 3	25 ± 1	31 ± 0		
Lys	16 ± 5	24 ± 15	34 ± 2	33 ± 4	13 ± 4	41 ± 1	28 ± 1	27 ± 1	33 ± 1	15 ± 19	37 ± 2	42 ± 1		

[a] Amino acids in tissue hydrolysate are listed according to the elution sequence of phenylthiocarbamyl derivatives separated by HPLC on a Waters Pico-Tag free amino acid column (3.9 × 3.0 cm). DW, dry weight. [b] Mean values and standard deviations are for pooled tissue samples; duplicate analyses were performed for each tissue. [c] No analysis was performed due to lack of sufficient tissue. [d] Asx and Glx represent asparagine + aspartate and glutamine + glutamate, respectively.

and indeed have lost much of their genomes during evolution (*27*), it is possible that they continue to function in metabolism by housing nuclear-encoded proteins such as EPSP synthase. In response to glyphosate treatment (0.25 kg ha^{-1}), individual amino acid concentrations of the parasite increased dramatically over that found in similarly treated host root tissue, which could be explained by either increased uptake of amino acids from the host or an increase in synthesis by the parasite, or both.

It is interesting to compare the relative differences in amino acid profiles between broomrape attachments growing on two different hosts. The parasite's amino acid composition was lower than that of the vetch root to which it is attached, but higher than that of rape root. This, perhaps, reflects a dependence of the parasite metabolism on that of the host. Certain amino acids such as Arg are clearly accumulated in the parasite, suggesting that the parasite is storing nitrogen. In related research, Arg was uniquely elevated in broomrape attachments compared to the host (carro, *Daucus carota* L.) root (*28*). An introduced annual root hemiparasite [*Parentucellia viscosa* (L.) Caruel] was found to accumulate certain amino compounds to a much greater or lesser relative extent than did its partner host(s) (*29*). This research raises several questions about the regulation of amino acid metabolism in broomrape and also demonstrates that glyphosate treatments, even at rates too low to cause visible injury to the host, alter amino acid profiles in both host and parasite. Investigations of enzymes involved in amino acid biosynthesis in broomrape will provide a better insight into nitrogen

regulation in the host−parasite interaction. Following the fate of ^{14}C-labeled or ^{15}N-labeled organic N substrates, Ala, for example, in host plants parasitized by broomrape should give some interesting insights into nitrogen metabolism by broomrape. Also, by selectively inhibiting the acetolactate synthase (ALS) enzyme of broomrape growing on ALS inhibitor-resistant hosts would provide additional insights into amino acid metabolic regulation in broomrape. ALS enzyme catalyzes the formation of the branched chain amino acids, valine, leucine, and isoleucine. Using a host-free growing system of broomrape such as callus cultures [as described by Ben-Hod et al. (*30*)] would be suitable for biochemical and physiological studies on broomrape amino acid regulation.

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