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DIFFERENT L-PHENYLALANINE POOLS AVAILABLE FOR THE BIOSYNTHESIS OF PHENOLICS IN BUCKWHEAT SEEDLING TISSUES

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Key Word Index—*Fagopyrum esculentum*; Polygonaceae; buckwheat: phenolic biosynthesis; protein catabolism; shikimic acid pathway; L-phenylalanine pool; flavonoids; L-phenylalanine ammonia-lyase; glyphosate; α-amino-oxyphenylpropionic acid.

Abstract—Using combined treatments with α-aminooxyphenylpropionic acid and glyphosate, the two inhibitors which selectively block deamination of L-phenylalanine and formation of aromatic amino acids via the shikimic acid pathway, respectively, quantitative estimates were made of the share of phenylalanine of different metabolic origin in the biosynthesis of phenolic compounds in buckwheat seedling tissues. A comparison of levels of free phenylalanine, shikimate, and flavonoids in the treated tissues indicated that in buckwheat hypocotyls the precursor pool available for phenolic synthesis consisted mainly of phenylalanine of primary origin arising directly from the shikimic acid pathway. By contrast, in buckwheat cotyledons more than a half of the total phenolics was apparently formed from phenylalanine of secondary origin released from proteins during their catabolism.

INTRODUCTION

While hardly anyone questions the precursor role of L-phenylalanine in the biosynthesis of most plant phenolics, little attention has been given to the fact that intracellular pools of protein amino acids including L-phenylalanine are heterogenous in metabolic origin. Consequently, phenolic compounds built up in a given tissue always consist of fractions of different metabolic origin. First, a portion of phenolics is synthesized from L-phenylalanine molecules which remain unused in proteins and undergo deamination by phenylalanine ammonia lyase (PAL) as soon as the molecules have been formed via the shikimic acid pathway. Second, a fraction of phenolic compounds is derived from L-phenylalanine of secondary origin, i.e. from those molecules of the amono acid which are released from proteins in the course of their catabolism. Ample indirect evidence is available that L-phenylalanine from both sources indeed can serve as a substrate for building plant phenolics [1–3].

The two fractions mentioned are completely identical chemically and there is no need, from that point of view, to place emphasis upon possible differences in the biochemical origin. However, from the physiological point of view, such differences cannot be neglected. If phenolic compounds of different plant tissues or some groups of phenolics within the same plant markedly differ in substrate origin then also their biosynthesis must have a different biological significance.

In this contribution an attempt was made to differentiate between L-phenylalanine of primary and secondary origin used in the formation of phenolics in buckwheat seedling tissues. Combined treatments with α-aminooxyphenylpropionic acid (AOPP) and glyphosate [*N*-(phosphonomethyl)-glycine] were used. As a result we are able to present here, for the first time, quantitative estimates characterizing the share of catabolic L-phenylalanine in the biosynthesis of phenolic compounds. Though these first estimates remain rather approximate, they still allow a preliminary insight into the problem and may serve to stimulate further experimental approaches in this field.

RESULTS

Theoretical premises of the experiments

The idea of using AOPP and glyphosate to differentiate between primary and secondary L-phenylalanine rests on the following theoretical considerations: (i) AOPP effectively inhibits the activity of PAL, the key enzyme of phenolic pathway [4]. It leads to a sharp reduction of phenolic biosynthesis and to a dramatic accumulation of free L-phenylalanine in the treated tissues [5]. The amount of L-phenylalanine so accumulated can serve as a tentative quantitative criterion of the total precursor pool of that amino acid available for phenolic synthesis. This pool includes L-phenylalanine of both primary and secondary origin.

(ii) Glyphosate specifically inhibits the activity of 5-enolpyruvylshikimate-3-phosphate synthase, one of the enzymes functioning in shikimic acid pathway [6, 7], and blocks, for that reason, synthesis of aromatic amino acids. When glyphosate is used in combination with AOPP then simultaneously channelling of L-phenylalanine into the phenolic pathway will be blocked. Accumulation of L-phenylalanine is expected to occur again but now the amino acid can originate solely from proteins. Thus, with these treatments the levels of free L-phenylalanine should be characteristic of the relative pool size of L-phenylalanine of secondary origin.

(iii) Glyphosate when used alone blocks primary formation of L-phenylalanine via shikimic acid pathway but

should probably leave untouched other metabolic reactions of the cell. A certain residual biosynthesis of phenolic compounds starting from protein L-pehnylalanine may therefore occur in the treated plants, providing an additional measure for estimating the role of the catabolic L-phenylalanine. Further, an accumulation of unmetabolized shikimic acid occurs in the glyphosate-treated plants [8, 9] and this may serve as a criterion of the total synthetic capacity of the pathway. It provides an indirect measure of the range of the overall production of L-phenylalanine of primary origin.

L-*Phenylalanine levels*

We first studied accumulation of L-phenylalanine in the treated tissues. AOPP, depending on the duration of the treatment, caused a three- to five-fold relative rise of free L-phenylalanine levels in cotyledons and a seven- to eight-fold rise of these levels in hypocotyls (Table 1). Maximum absolute increases reached about 90 and 140 nmol of L-phenylalanine per seedling in cotyledons and hypocotyls, respectively (Fig. 1). More prolonged treatments, in general, led to the further accumulation of free L-phenylalanine.

Glyphosate used alone did not cause any significant change in the normal low level of free L-phenylalanine (Table 1). However, when used in combination with AOPP it markedly reduced L-phenylalanine accumulation typical of AOPP-treated tissues. In the hypocotyls, glyphosate, in fact, almost totally nullified the effect of AOPP (Table 1, Fig. 1). Thus in buckwheat hypocotyls the precursor pool for building phenolics is mainly, if not exclusively, composed of primary L-phenylalanine originating directly from the shikimic acid pathway.

In the cotyledons considerable amounts of free L-phenylalanine accumulated in the presence of both inhibitors (Fig. 1). The absolute levels under these conditions corresponded to about 60% of those levels which were found in the cotyledons treated with AOPP alone. Hence in buckwheat cotyledons more than a half the total phenolic compounds may be synthesized from L-phenylalanine of secondary origin released from proteins during catabolism.

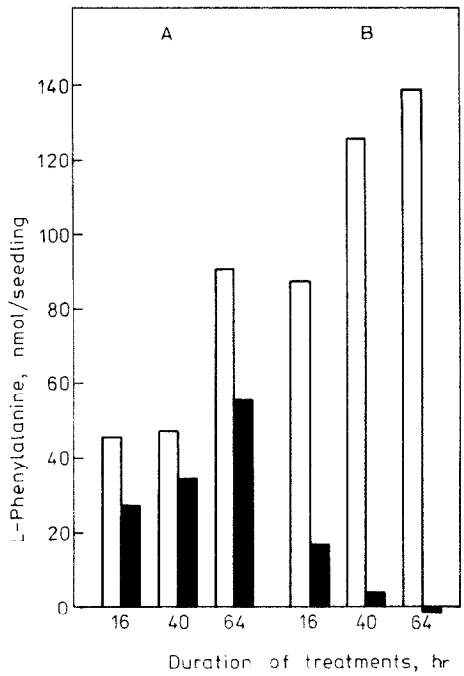

Fig. 1. Accumulation of free L-phenylalanine in the illuminated buckwheat cotyledons (A) and hypocotyls (B) treated for various time periods with 1 mM AOPP alone (white bars) or with 1 mM AOPP in combination with 10 mM glyphosate (black bars). The difference between the two treatments, as estimated by the analysis of variance, is statistically significant at 95% confidence level (cotyledons: $F = 15.4$; d.f. $= 1/22$; hypocotyls: $F = 97.8$; d.f. $= 1/22$).

Table 1. Free L-phenylalanine levels* in excised buckwheat hypocotyls and cotyledons as affected by a 16, 40 or 64 hr incubation in the presence of AOPP (1 mM) and glyphosate (10 mM) in light

Duration of incubation in the light (hr)	Control (H_2O), nmol/seedling	L-Phenylalanine levels† % of the control		
		AOPP	Glyphosate	AOPP + glyphosate
Hypocotyls				
16	15.3	668	100	208
40	17.4	820	89	123
64	19.8	800	87	92
Cotyledons				
16	18.3	346	82	247
40	20.0	334	117	267
64	24.6	467	119	324

*Average data of five independent experiments.

†AOPP effects highly significant, glyphosate effects not significant at 95% confidence level as estimated by Student's *t*-test; combined effects of AOPP and glyphosate significant in cotyledons only but not significant in hypocotyls except a series with 16 hr incubation.

Shikimic acid levels

This inference can be considered correct provided that AOPP, apart from its role in blocking PAL activity, does not interfere with the biosynthesis of L-phenylalanine via shikimic acid pathway. To check this, we compared shikimic acid accumulation in buckwheat cotyledons and hypocotyls treated with glyphosate alone or in combination with AOPP.

In buckwheat cotyledons total blockage of the shikimic acid pathway by glyphosate, in a 20 hr treatment, can be attained with a 0.1 mM solution of the herbicide in the light and with a 1 mM solution in the dark [10]. Hence, the more concentrated solution of glyphosate used here (10 mM) had to be powerful enough to stop production of primary L-phenylalanine at the very beginning of the treatment period. High doses of glyphosate, nevertheless, did not disturb functioning of the aromatic pathway prior to the reaction catalysed by 5-enolpyruvylshikimate-3-phosphate synthase. The amount of shikimic acid in a 10 mM solution of glyphosate remained virtually the same as in the solutions of lower concentrations (Table 2) and its rate of accumulation was almost unchanged during at least a 64 hr period of incubation of cotyledons and hypocotyls in the presence of the herbicide (Fig. 2; see also [10]).

These data showed that shikimic acid levels in glyphosate-treated tissues can be considered a reliable criterion for characterizing the potency of the aromatic pathway. Thus, in buckwheat cotyledons we could estimate this potency to be about 450 and 700 nmol of shikimic acid equivalents per seedling during a 40 and 64 hr period of incubation, respectively, while in the hypocotyls the corresponding estimates were about 290 and 430 nmol/seedling (Fig. 2). In the absence of light, the synthetic capacity of the shikimic acid pathway was markedly reduced.

Surprisingly enough, AOPP considerably reduced the accumulation of shikimic acid in the glyphosate-treated tissues, especially in the cotyledons (Fig. 2). This was obviously due either to a direct but so far unknown inhibitory action of AOPP on the enzymes that catalyse the initial steps of aromatic biosynthesis prior to formation of shikimate-3-phosphate intermediate or by a feedback inhibition of the same enzymes caused by excess levels of accumulated L-phenylalanine. In the latter case the mechanism of feedback inhibition, however, should be different from that proposed for control of the shikimate-pathway systems by Jensen [11, 12].

Decreased levels of shikimic acid in tissues incubated in the presence of AOPP unambiguously suggest that the L-phenylalanine data in such experiments need to be corrected before they can be employed for quantitative comparisons. After making necessary corrections, the levels of free L-phenylalanine in AOPP-treated tissues graphically presented in Fig. 1 are as shown in Table 3. From these corrected data, it is clear that in buckwheat cotyledons the share of catabolic L-phenylalanine in the total precursor pool for building phenolics constituted *ca* 53%, on average.

Proceeding from shikimic acid levels in glyphosate-treated tissues (Fig. 2) and using normal ratios of the three aromatic amino acids (Table 4) as a matrix for calculations, it was possible to make tentative estimates of the production of L-phenylalanine in buckwheat seedling tissues. For a standard 40 hr period of incubation, this could reach a maximum of 170 and 100 nmol/seedling roughly in the cotyledons and hypocotyls, respectively.

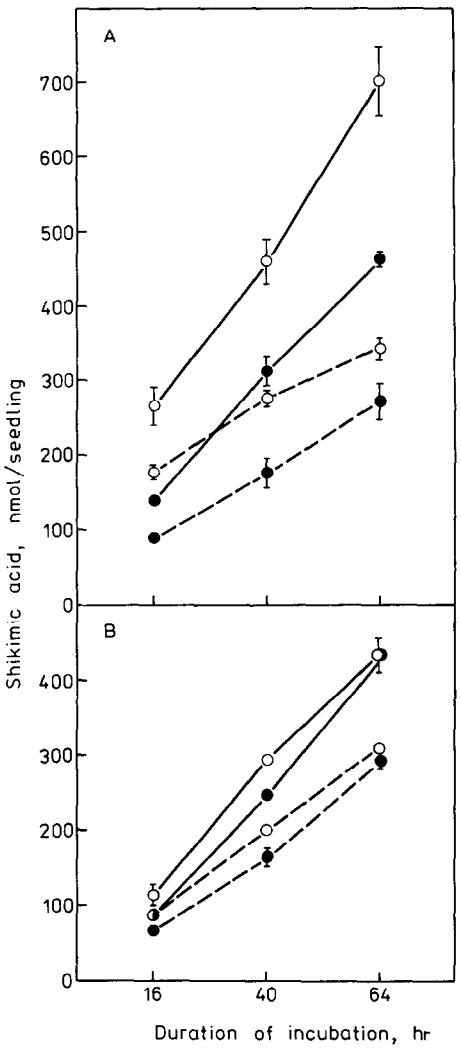

Fig. 2. Shikimic acid accumulation in the excised buckwheat cotyledons (A) and hypocotyls (B) incubated for various time periods in a 10 mM solution of glyphosate (open symbols) or in a mixed solution of 10 mM glyphosate and 1 mM AOPP (closed symbols) in the light (solid curves) or in darkness (broken curves). Vertical bars denote mean errors.

Table 2. Shikimic acid levels in illuminated buckwheat cotyledons incubated for 40 hr in the presence of various doses of glyphosate

Glyphosate concentration (mM)	Shikimic acid (nmol/seedling)
0.1	705
1	696
10	714

Table 3. Free L-phenylalanine levels* in the AOPP-treated buckwheat tissues corrected according to the partial inhibition of the aromatic biosynthesis caused by AOPP

Duration of treatment in the light (hr)	L-Phenylalanine (nmol/seedling)		L-Phenylalanine levels in AG, % of its levels in A
	AOPP (A)	AOPP + glyphosate (AG)	
Cotyledons			
16	62.3	27.0	43.3
40	53.1	33.8	63.7
64	108	55.2	51.0
Hypocotyls			
16	109	16.6	15.2
40	149	4.05	2.7
64	139	0	0

*Calculated from the data graphically shown in Fig. 1 on the basis of the AOPP-caused inhibition of shikimic acid accumulation shown in Fig. 2.

Table 4. Free aromatic amino acid levels* in illuminated buckwheat cotyledons and hypocotyls incubated in water

Duration of incubation (hr)	Aromatic amino acids, nmol/seedling			L-Phenylalanine, % of the sum of the three aromatic amino acids
	L-Phenylalanine	L-Tyrosine	L-Tryptophan	
Cotyledons				
16	18.3	3.10	52.7	24.7
40	20.0	3.49	30.6	37.0
64	24.6	4.24	22.5	48.0
Hypocotyls				
16	15.3	8.90	16.0	38.1
40	17.4	8.45	23.9	34.9
64	19.8	8.05	18.1	43.0

*Average of five independent experiments.

Flavonoid levels

Although increasing doses of AOPP suppressed flavonoid biosynthesis in buckwheat cotyledons, a treatment even with a 1 mM solution proved insufficient to inhibit that process completely. As can be seen from a typical set of flavonoid data (Table 5), at that high concentration of the inhibitor *ca* 15% of the total synthesizing capacity of the cotyledons still remained unblocked. In the hypocotyls AOPP inhibited flavonoid (anthocyanin and rutin) biosynthesis more effectively but also in that tissue an accumulation of small amounts of these compounds up to a 10% level of their normal production was generally observed after a treatment with a 1 mM solution of the inhibitor (data not shown). From this it follows that increased levels of free L-phenylalanine in the AOPP-treated buckwheat tissues actually corresponds to a 85–90% blockage of phenolic synthesis.

Glyphosate was not expected to completely inhibit flavonoid biosynthesis but to allow a continuation of that process at the expense of the catabolic L-phenylalanine (see above). In a series of experiments designed to test the effects of various doses of the herbicide on the cotyledons a residual production of these compounds was observed, indeed (Table 6). However, the amount of flavonoids produced proved to be less than it could be anticipated from the pertaining L-phenylalanine data (cf. Fig. 1 and

Table 5. Influence of AOPP on the production of flavonoids in excised buckwheat cotyledons during a 40 hr incubation under continuous illumination

AOPP concentration, (M)	Flavonoids*, nmol/seedling	% of the control
0 (H_2O)	447	100
10^{-7}	413	92.3
10^{-6}	451	101
10^{-5}	333	74.5
10^{-4}	137	30.6
10^{-3}	70.0	15.7

*Sum of vitexin, isovitexin, orientin, *iso*-orientin, rutin, and anthocyanins.

Table 3). Moreover, in repeated experiments with 10 mM glyphosate, the formation of flavonoids was often found to be entirely stopped. Since enough substrate was available under these conditions, the reason for this probably was either a glyphosate-induced inhibition of the activity of enzymes involved in the biosynthesis of flavonoids or a rapid decomposition of some of these enzymes (i.e. phenylalanine ammonia-lyase [13, 14]) that, due to inhibition of the aromatic amino acid pathway, could not be compensated for by the synthesis of new enzymic proteins.

In some cotyledon samples, a significant production of flavonoids was still observed in the glyphosate-treated tissues with the total amount of these compounds produced reaching, in extreme cases, *ca* 25% in the light and nearly 50% in the dark as compared with their production in the untreated cotyledons (Fig. 3). Thus, relative flavonoid levels were approximately of the same order of magnitude as were relative L-phenylalanine levels found in the cotyledons after their combined treatment with AOPP and glyphosate (cf. Fig. 3 and Table 3). Flavonoid production in this case reflects, as expected, synthesis of these compounds from L-phenylalanine of protein origin.

In hypocotyls treated with a 10 mM solution of glyphosate, no significant production of flavonoids was observed (data not shown). This was in a good agreement with the data on the accumulation of L-phenylalanine (see Table 3).

Table 6. Influence of glyphosate on the production of flavonoids in excised buckwheat cotyledons during a 40 hr time period of incubation (16 hr in the light + 24 hr in darkness)

Glyphosate concentration, (M)	Flavonoids*, nmol/seedling	% of the control
0 (H_2O)	420	100
10^{-4}	163	38.8
10^{-3}	87.8	20.9
10^{-2}	45.2	10.8

*Sum of vitexin, isovitexin, orientin, *iso*-orientin, and rutin. Mean values of two independent experiments.

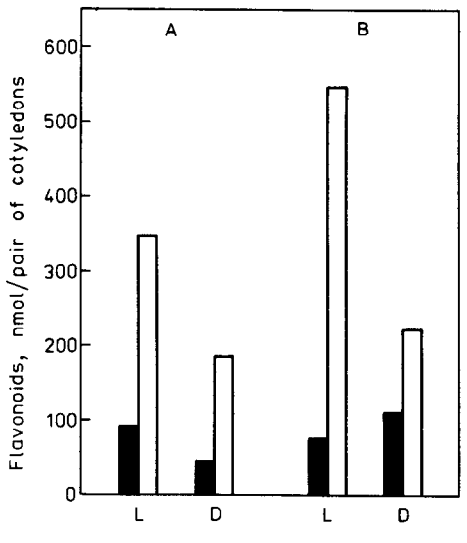

Fig. 3. Production of flavonoids (vitexin, isovitexin, orientin, isoorientin, and rutin) in a sample of buckwheat cotyledons which showed increased resistance against damaging influence of glyphosate. (A) material incubated in the presence of 10 mM glyphosate for 16 hr, (B) material incubated in the same solution for 40 hr. Black bars—treated material, white bars—controls; (L) incubation in the light, (D) incubation in darkness.

PAL activity

Extractable PAL activity of cotyledons treated with glyphosate showed a maximum decrease of *ca* 50–55% as compared with the untreated material (Table 7). However, such a level of inhibition was not typical but developed in that tissue only as a result of prolonged treatments with the herbicide. It indicated that an impairment of PAL catalytic functions could hardly be responsible for the severe curtailing effect of glyphosate on the production of flavonoids observed in cotyledons. In hypocotyls changes in PAL activity obviously did not play a role at all: in that tissue a treatment with glyphosate even brought about a marked increase in the extractable activity of the enzyme (Table 7).

In the case of treatments with AOPP, extractable activity of PAL in vitro does not correspond to the deaminating capacity of tissues *in vivo* [15, 16]. It is still interesting to note that enzyme extracts from both cotyledons and hypocotyls incubated in the presence of AOPP for a comparatively short time period (16–24 hr) showed markedly lower levels of PAL activity while after more prolonged incubations the activity gradually grew to much higher values which even exceeded PAL activity levels of the controls (Table 7).

DISCUSSION

The results obtained clearly show that the composition of the endogenous pool of L-phenylalanine available for building phenolic compounds may really be different in different plant tissues.

In buckwheat hypocotyls the pool seems to be composed mainly of L-phenylalanine originating directly from shikimic acid pathway: after a combined treatment with AOPP and glyphosate the tissue did not accumulate extra amounts of free L-phenylalanine or did so to a very limited extent (in result of a 16-hr incubation, see Fig. 1).

This conclusion is strongly corroborated by the fact that the increase in the content of free L-phenylalanine actually found in the AOPP-treated hypocotyls (120–140 nmol/seedling, see Fig. 1) was in a good harmony with the production of phenolic compounds normally characteristic of the untreated hypocotyls during a comparable time period (about 110–140 nmol/seedling; see [17, 18]). True, the calculated estimate of the potency of buckwheat hypocotyls in producing L-phenylalanine via shikimic acid pathway proved to be somewhat smaller of that level (about 100 nmol/seedling per a 40 hr period of incubation). It must be taken into account, however, that alternative transformations of shikimic acid via metabolic routes other than the aromatic pathway [19] can be elevated in the case of its substantial accumulation. Functional capacity of the pathway as measured by the increased shikimic acid levels in the glyphosate-treated tissues may thus remain underestimated.

Table 7. Extractable PAL activity in illuminated buckwheat cotyledons and hypocotyls treated for various time periods with glyphosate (10 mM) or with AOPP (1 mM)

Duration of treatment (hr)	PAL activity, % of the control			
	Glyphosate		AOPP	
	Cotyledons	Hypocotyls	Cotyledons	Hypocotyls
Experiment No. 1 (72-hr-old material):				
24	95	147	24	48
48	51	176	60	150
72	44	165	142	185
96	61	126	187	210
Experiment No. 2 (80-hr-old material):				
16	102	158	12	27
40	94	266	31	97
64	70	211	60	161
88	63	—	136	179

In buckwheat cotyledons marked accumulation of free L-phenylalanine was observed not only after treatment with AOPP but also in result of a combined treatment with AOPP and glyphosate, i.e. it occurred also in the tissues having the aromatic pathway completely blocked (see also [18, 20]). Since under these conditions L-phenylalanine molecules could come solely from proteins (see also [21]), the conclusion can be drawn that the cotyledonary precursor pool for building phenolics is essentially different, by its metabolic origin, from a similar pool in buckwheat hypocotyls and is composed mostly of L-phenylalanine released from proteins during catabolism.

The absolute amount of accumulated L-phenylalanine in the AOPP-treated cotyledons remained several times lower than could be expected by virtue of the true inhibition of flavonoid biosynthesis (cf. Tables 3 and 5). Formation of other phenolics was probably blocked too. Hence it appears that in cotyledons a major part of L-phenylalanine which could not be channelled into the phenolic pathway either was bound into complexes with other cell metabolites (excluding its transfer into the free pool) or underwent some kind of metabolic transformation which did not require removal of the amino group by PAL reaction [22, 23]. Free pool data of L-phenylalanine could thus only then be representative of the composition of its total pool for phenolic synthesis if no selective or preferential accumulation of either primary or secondary L-phenylalanine occurred in the AOPP-treated tissues.

Unfortunately the data on the accumulation of flavonoids in the glyphosate-treated cotyledons are unreliable as a measure of the catabolic L-phenylalanine involved in the biosynthesis of phenolics. More informative for this purpose were shikimic acid data and calculated estimates of the range of L-phenylalanine primary production. For buckwheat cotyledons the corresponding calculations yielded an estimate of 170 nmol/seedling for a 40 hr period of incubation. However, total production of phenolics in that tissue within the same time period was at least 600–650 nmol/seedling (see Table 6 and [17]). From a comparison of these two figures, it should be readily apparent that in the cotyledons the potency of the shikimic acid pathway remained insufficient to satisfy precursor requirements for the biosynthesis of phenolics because aromatic amino acids were also needed for protein synthesis. It is not inconceivable that more than a half of all phenolic compounds of buckwheat cotyledons was indeed formed from secondary L-phenylalanine of protein origin, as inferred from the accumulation of free L-phenylalanine in the AOPP- and (AOPP + glyphosate)-treated tissues.

EXPERIMENTAL

Plant material. The experiments were carried out with isolated buckwheat (Fagopyrum esculentum Moench) cotyledons and hypocotyls excised from 72–80 hr etiolated seedlings grown in H_2O. The excised material was transferred to a phytotron and incubated there for 16–64 hr on filter paper moistened with the following solutions: (1) glyphosate, 10 mM; (2) AOPP, 1 mM; (3) a mixture of glyphosate (10 mM) and AOPP (1 mM). In the control, the material was incubated in dist. H_2O. Standard incubation conditions: continuous illumination from white fluorescent tubes, light intensity 28 W/m^2, temp. 25°. In some experiments, another portion of the treated material was incubated in the dark. In all cases the material was assayed in the fresh state immediately after incubation.

Analytical methods. The content of free L-phenylalanine and other amino acids was determined by a standard procedure using a Microtechna amino acid analyser T-339.

The content of individual flavonoids (rutin, orientin, isoorientin, vitexin, and isovitexin) in the cotyledons was determined spectrophotometrically after their paper chromatographic separation as described elsewhere [24, 25].

The content of shikimic and quinic acids in glyphosate-treated material was determined by a method of ref. [10]. The material was extracted with dilute EtOH, the extract evapd to dryness at room temp., and the dry residue dissolved in a small vol. of dist. H_2O. The resulting solution was applied to a column of polyamide (15 × 15 mm) followed by elution with dist. H_2O. The first 2 ml of the effluent were discarded. The next 16 ml containing shikimic and quinic acids were collected and then chromatographed by ascent on a Filtrak FN-paper in *n*-BuOH–HOAc–H_2O (4:1:1). The dried chromatograms were

first sprayed with fresh 0.03 M Na_3HIO_6 in 0.2 M HOAc acid [26, 27] and thereafter developed, after the first spray had dried, with a fresh soln of Na-nitroprusside (0.5 g) and piperazine adipinate (0.5 g) [28] in dil. EtOH acidified with HOAc (total vol. 70 ml). On the sprayed chromatograms both shikimic and quinic acids appeared as distinct yellow spots. The spots were eluted with dist. H_2O and the absorbance of the eluates measured at 431 nm. On each chromatogram, parallel to aliquots of plant extracts, standard amounts of the authentic shikimic and quinic acids were run and developed. The absorbances of the eluates of the standards were used as the basis for final calculations.

As in almost all plant samples quinic acid levels proved to be near the lowest limit of assay and showed no tendency of changing in a different manner than were the changes in the content of shikimic acid, the experimental data on that acid are not shown or discussed.

Enzyme assay. The activity of L-phenylalanine ammonialyase (PAL) was determined by the amount of cinnamic acid formed from L-phenylalanine in an assay mixture during its incubation for 4 hr at 35°. The assay mixture consisted of 0.1 M borate buffer (2.7 ml; pH 8.8), 0.01 M L-phenylalanine (0.1 ml), and a sample (0.2 ml) of enzyme extract prepared using 0.1 M borate buffer for homogenization of plant material [29, 30].

Statistical evaluation of data. The experiments, as a rule, were run in 3–5 replicate series. The results were subjected to statistical evaluation by Student's significance test and by analysis of variance.

Chemicals. Glyphosate was a Soviet 36% commercial preparation for herbicidal use. AOPP was synthesized by V. Hodorkovski (Department of Organic Chemistry of the Riga Polytechnical Institute). Standards of shikimic and quinic acids were generous gifts of V. Osipov (Krasnoyarsk Forest and Wood Institute). Other chemicals were Soviet commercial preparations of analytical grade.

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