JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

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Role of Exogenously Supplied Ferulic and *p*-Coumaric Acids in Mimicking the Mode of Action of Acetolactate Synthase Inhibiting Herbicides

Luis Orcaray, María Igal, Ana Zabalza, and Mercedes Royuela*

Departamento de Ciencias del Medio Natural, Universidad Pública de Navarra, Campus Arrosadia, E-31006 Pamplona, Spain

ABSTRACT: Chlorsulfuron and imazethapyr (herbicides that inhibit acetolactate synthase; ALS, EC 4.1.3.18) produced a strong accumulation of hydroxycinnamic acids that was related to the induction of the first enzyme of the shikimate pathway, 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase (EC 2.5.2.54). The exogenous application of two hydroxycinnamic acids, ferulic and *p*-coumaric acids, to pea plants resulted in their internal accumulation, arrested growth, carbohydrate and quinate accumulation in the leaves, and the induction of ethanolic fermentation. These effects resemble some of the physiological effects detected after acetolactate synthase inhibition and suggest important roles for ferulic and *p*-coumaric acids in the mode of action of herbicides inhibiting the biosynthesis of branched chain amino acids.

KEYWORDS: Acetolactate synthase, branched chain amino acid, mode of action, hydroxycinnamic acid, secondary metabolism

INTRODUCTION

The aromatic amino acids phenylalanine, tryptophan, and tyrosine are the final products of the shikimate pathway. In higher plants, these aromatic amino acids have a dual purpose, serving as both substrates for protein synthesis and as precursors for the synthesis of a vast array of secondary metabolites, such as hydroxycinnamic acids, which are the precursors of lignin and other phenolic acids. The first enzyme in the shikimate pathway is 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase (DAHPS; EC 2.5.2.54), which catalyzes the condensation of phosphoenolpyruvate and erythrose-4-phosphate to 3-deoxy-D-arabino-heptulosonate 7-phosphate. The L-phenylalanine ammonia lyase (PAL; EC 4.3.1.5) enzyme links the flow of metabolites between primary and secondary metabolism, catalyzing the deamination of phenylalanine to yield *trans*-cinnamic acid, which is the common precursor for the biosynthesis of phenolic derivatives.

The imidazolinones and the sulfonylureas are two classes of herbicides with a common mechanism of action: inhibition of acetolactate synthase (ALS, also known as acetohydroxyacid synthase; EC 4.1.3.18). ALS is the first common enzyme in the biosynthesis of branched chain amino acids: valine, leucine, and isoleucine. The discovery of the well-established mechanism of action of both type of herbicides was almost simultaneous.[1,2]

The mode of action of imidazolines and sulfonylureas has not been completely elucidated, although several physiological effects are well-known. These herbicides affect carbon metabolism by decreasing the sink strength of the roots, which causes carbohydrate accumulation in the leaves of treated plants. It has also been reported that a metabolic alteration, which is probably related to an imbalance in amino acid metabolism, impairs the carbohydrate utilization by the roots of plants treated with ALS inhibitors.[3] Moreover, besides not consuming available carbohydrates, which in fact accumulate, several futile pathways, pyruvate decarboxylase (EC 4.1.1.1; PDC) and alcohol dehydrogenase (EC 1.1.1.1; ADH), in the ethanolic fermentative pathway[4] and alternative respiratory pathway[5] are induced in these roots.

More recently, it has been reported that herbicides that inhibit branched chain amino acid biosynthesis affect the shikimate pathway. Quinate, which is a metabolite synthesized in a lateral branch of the shikimate pathway, is accumulated in the leaves of herbicide-treated plants.[6] In addition to the shikimate pathway, previous studies have also shown an increase in the secondary metabolites that are derived from aromatic amino acids in sulfonylurea-treated plants and has been detected by an increase in PAL activity and hydroxycinnamic acid content.[7−9] Treatment with sulfonylurea herbicides induced a strong accumulation of *p*-coumaric acid, caffeic acid, ferulic acid, and synapic acid in sunflower hypocotyls.[8] Nevertheless, it remains to be established whether the induction of secondary metabolism is a general effect of ALS inhibitors independently of the herbicide's chemical class (sulfonylurea or imidazolinone) or it is a specific effect of sulfonylurea herbicides.

Plants release organic compounds into the environment, which both positively and negatively influence the growth and development of neighboring plants via a phenomenon called allelopathy that is mediated by allelochemicals.[10] A large number of compounds are considered allelochemicals. Among them, the hydroxycinnamic acids, *p*-coumaric acid and ferulic acid, are well-known allelochemical agents[11] that mainly inhibit the growth of plants.[12] Because after ALS inhibition by sulfonylureas *p*-coumaric acid and ferulic acid accumulation,[9] it is possible that the growth inhibition that is detected after herbicide application may be related to hydroxycinnamic acid accumulation. To evaluate whether *p*-coumaric acid and ferulic acid mediate the toxic effects of the herbicides or even can mimic their action, the exogenous application of both hydroxycinnamic acids should be compared with the effects of herbicidal treatment.

Received: June 28, 2011
Accepted: August 26, 2011
Revised: August 24, 2011
Published: August 26, 2011

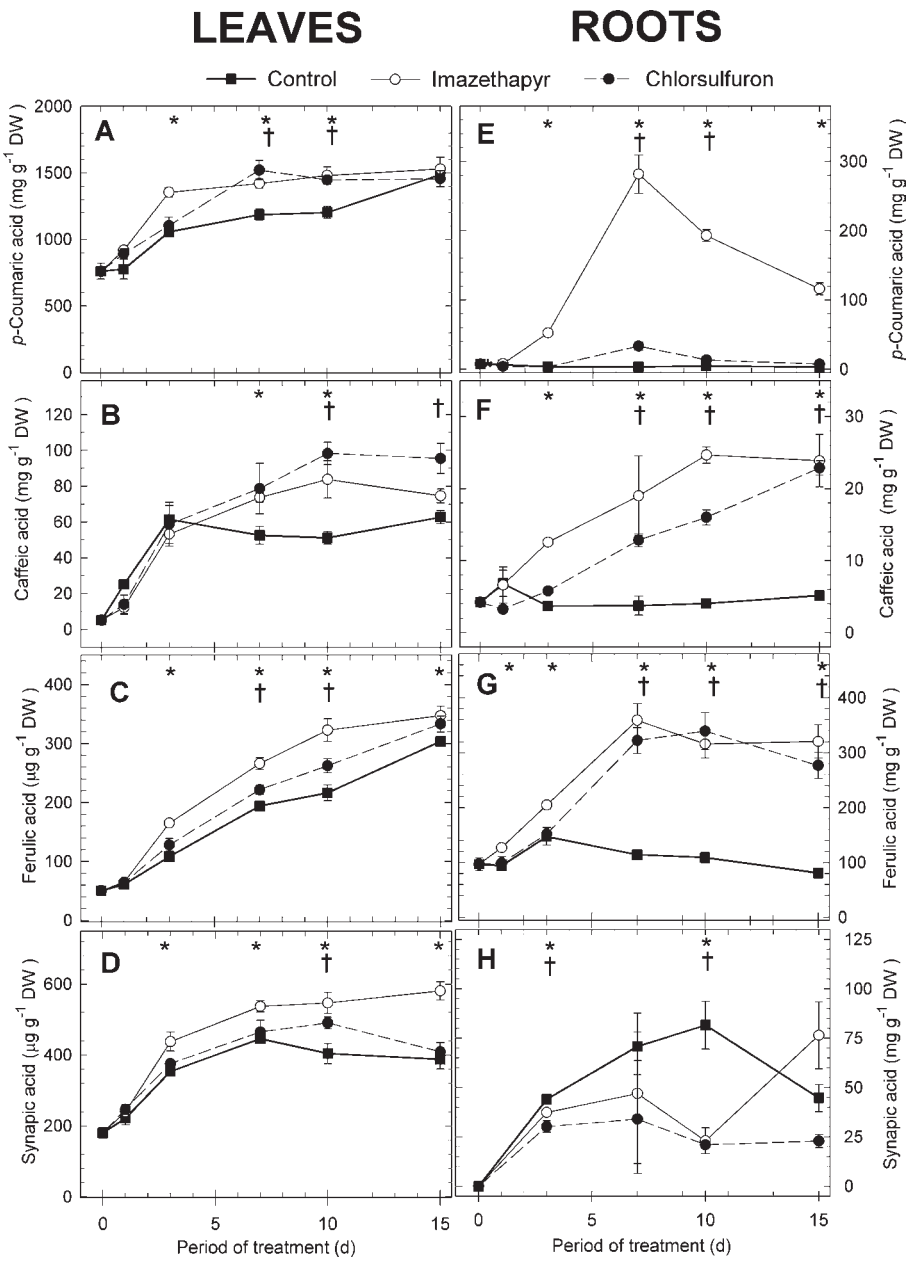

Figure 1. *p*-Coumaric (A and E), caffeic (B and F), ferulic (C and G), and synapic (D and H) acid content in the leaves (A−D) and roots (E−H) of control pea plants or plants treated with imazethapyr or chlorsulfuron. Each value is the mean ± standard error (n = 5). Symbols indicate significant differences between the control and imazethapyr (*) or the control and chlorsulfuron (†) on a given day ($p < 0.05$).

The first objective of the study presented here was to ascertain whether there was a general pattern of hydroxycinnamic acid content (*p*-coumaric acid, caffeic acid, ferulic acid, and sinapic acid) following ALS inhibition independently of the herbicide chemical class. For this purpose, the hydroxycinnamic acid content and DAHPS were studied in pea plants treated with two classes of ALS inhibitors, chlorsulfuron (a sulfonylurea) and imazethapyr (an imidazolinone). We detected a strong accumulation of *p*-coumaric acid and ferulic acid after the application of both types of herbicides. These findings led us to investigate the role of these compounds in the mode of action of the herbicides by exogenously applying ferulic or *p*-coumaric acid to pea plants and studying their possible effects on carbon metabolism and quinate content.

MATERIALS AND METHODS

Materials and Instrumentation. Commercial imazethapyr (Pursuit) was supplied by BASF SA (Barcelona, Spain), and commercial chlorsulfuron (Glean) was supplied by Dupont (Dupont Ibérica SA, Barcelona, Spain). All of the other reagents were obtained from Sigma Chemical Co. (St. Louis, MO).

High-performance liquid chromatography (HPLC) detection was performed using a Waters 2690 Separation Module with a Waters 2487 UV detector (Waters, Milford, MA). Capillary electrophoresis was conducted using a P/ACE system 5500 (Beckman Instruments, Fullerton, CA). Ion chromatography was performed using a DX-500 system (Dionex Corporation, Sunnyvale, CA). All of the spectrophotometric determinations used a Synergy HT microplate reader (Biotek

Instruments, Inc., VT). Polyacrylamide gels were electrophoresed using the Mini-Protean 3 system (Bio-Rad, Hercules, CA).

Plant Material and Treatment Application. Pea plants (*Pisum sativum* L. cv. Snap Sugar Boys) were grown as previously described.[4] In total, 69 μM (20 mg/L active ingredient) imazethapyr, 28 nM (10 μg/L active ingredient) chlorsulfuron, 1 mM ferulic acid, or 1 mM *p*-coumaric acid were applied to the nutrient solution when the plants were 12 days old. The nutrient solution was replaced every 3 days. No one of the concentrations tested modified the pH of the nutrient solution significantly. With or without products, the pH of the nutrient solution was maintained between 6 and 7 for the whole period of treatment.

Plant samples were collected at 0, 1, 3, 7, 10, and 15 days. In indicated cases, the study only included the harvest at day 7 or day 15. At harvest, samples were taken, immediately frozen in liquid nitrogen, and stored at −80 °C for further analytical determinations.

Hydroxycinnamic Acid Determination. The *p*-coumaric acid, caffeic acid, ferulic acid, and sinapic acid content was assessed using HPLC, as previously described.[6]

Carbohydrate Determination. The glucose, fructose, sucrose, and starch content were determined using capillary electrophoresis, as previously described.[3]

Quinate Content. Quinate determination in pea leaves was measured using ion chromatography as described previously.[6]

PAL Assay. The PAL activity was determined in pea roots, using methods previously described[13] with the following modifications: No filtration using Sephadex G 25 columns was performed, the reaction proceeded for 1 h at 37 °C, and after the addition of HCl, the mixture was centrifuged at 5000*g* for 5 min.

Lignin Content. The lignin content was determined in pea roots according to previously described methods.[13]

DAHPS, PDC, ADH, and Western Blot Analysis. DAHPS Western blots were produced using standard techniques. DAHPS antibody was produced by a custom peptide facility (Biogenes, Berlin, Germany) using a short, conjugated peptide as an antigen (C-QFAKPR-SDSFEEKN). The antibody was raised in rabbits using standard protocols from the manufacturer, and the primary antibody dilution was 1:1000. PDC and ADH Western blots were performed, as previously described.[14]

Statistical Analysis. Findings were examined using the one-way analysis of variance. Each mean value was calculated using samples from different single plants as replicates. Significant differences between each treatment and control plants (untreated plants) are highlighted in the figures with different symbols for each treatment [($p < 0.05$) on a given day of treatment by the Fisher test].

RESULTS AND DISCUSSION

The supply of 69 μM imazethapyr or 28 nM chlorsulfuron caused similar effects on pea growth when added to the nutrient solution and caused plant death 22−23 days after the onset of the treatment. These effects on pea growth have been previously published.[3,4] Although the two herbicides belong to different chemical classes, the similar physiological effects are consistent with the effects of ALS inhibition as the common mechanism of action.

The contents of hydroxycinnamic acid in the leaves and roots were significantly affected by both herbicides (Figure 1). The leaves of treated plants showed an accumulation of *p*-coumaric acid, caffeic acid, ferulic acid, and sinapic acid. However, different patterns for each acid were suggested after the imazethapyr or chlorsulfuron treatment. The sinapic acid content in treated roots was significantly reduced after chlorsulfuron or imazethapyr treatment, as compared with those in the control roots at days 3 and 10 from the onset of the treatment (Figure 1H),

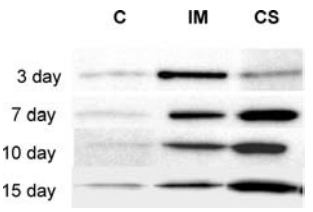

Figure 2. Immunoblots of DAHPS in roots of control (C) pea plants or those treated with imazethapyr (IM) or chlorsulfuron (CS) 3, 7, 10, or 15 days after treatment. Each lane contains 40 μg of protein.

whereas the *p*-coumaric acid, caffeic acid, and ferulic acid contents in the roots of imazethapyr- and chlorsulfuron-treated plants were significantly increased throughout the experimental period (Figure 1E−H). The results indicate that ALS inhibition induces the accumulation of hydroxycinnamic acids, which has been previously reported for sulfonylureas.[8,9] In addition, the accumulation of hydroxycinnamic acids was higher in the roots in comparison with that in the leaves of treated plants.

The detected induction of secondary metabolites that were derived from aromatic amino acids suggested a coordinated response of the shikimate pathway. To evaluate the possibility of a higher carbon flow through the shikimate pathway, the effect of herbicides on the amount of DAHPS protein was evaluated. As expected, the induction of secondary metabolism after the treatment with ALS inhibitors was associated with an induction of the first enzyme of the shikimate pathway in pea roots (Figure 2). In plants, DAHPS is subject to regulation by developmental and other environmental factors, such as abiotic stresses. Exposure of potato cells to the herbicide glyphosate or wounding showed increased DAHPS protein levels and activity.[15] Moreover, several other studies have revealed that the plant response to an environmental stimulus, which includes an increase in secondary metabolites, is associated with an increase in DAHPS activity.[16−18]

After the effect of chlorsulfuron and imazethapyr treatments on the content of several hydroxycinnamic acids was determined, we investigated the possible role of *p*-coumaric acid and ferulic acid in the mode of action of ALS inhibitors. Commercially available ferulic acid and *p*-coumaric acid were included in the nutrient solution of pea plants, and their effects were compared with those reported after herbicidal treatment. The objective was to verify whether these compounds mediated the toxic effects of the herbicides or even can mimic their action. On the basis of previously reported doses,[19] 1 mM *p*-coumaric or ferulic acid was used. After the *p*-coumaric acid and ferulic acid concentrations in the nutrient solution were monitored during the experiments, we established that refreshing the nutrient solution every 3 days was sufficient to minimize microbial contamination and to maintain hydroxycinnamic acid availability.

The supplementation of 1 mM *p*-coumaric acid or ferulic acid led to a dramatic increase of these compounds in the roots, and the levels of *p*-coumaric acid (Figure 3D) and ferulic acid (Figure 3B) were similar to or higher than those detected after ALS inhibition. These findings validate other studies comparing the physiological effects of *p*-coumaric acid and ferulic acid. The accumulation of *p*-coumaric acid and ferulic acid was also detected in the leaves (Figure 3A,C), demonstrating that *p*-coumaric acid and ferulic acid were absorbed by roots and translocated to the leaves.

The abilities of supplemented ferulic acid or *p*-coumaric acid to inhibit pea growth were similar. Root elongation was arrested

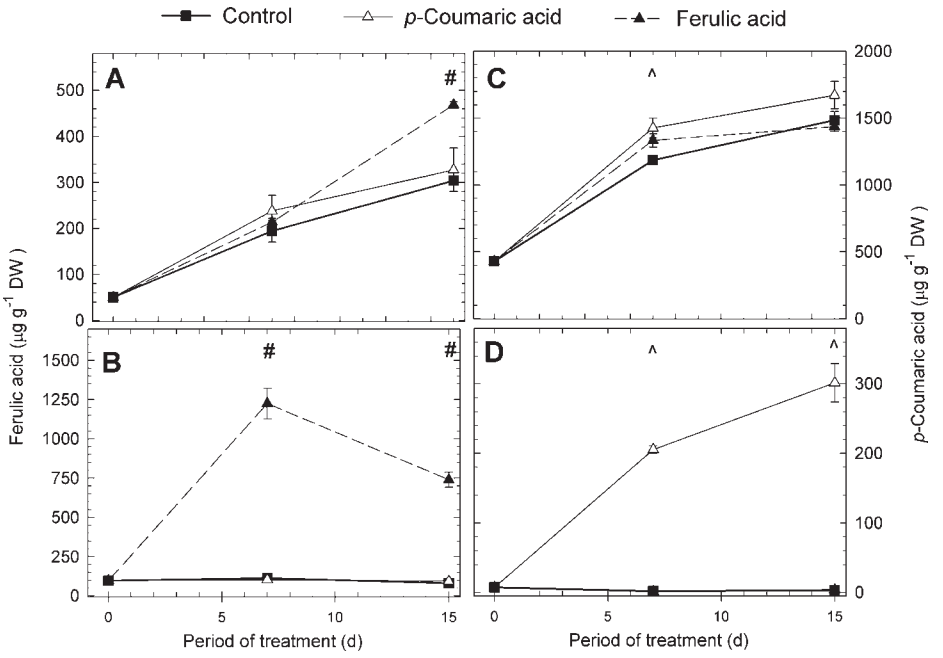

Figure 3. Ferulic acid (A and B) and *p*-coumaric (C and D) content in the leaves (A and C) and roots (B and D) of control pea plants or those treated with ferulic acid or *p*-coumaric acid supplied in the nutrient solution. Each value is the mean ± standard error (n = 5). Symbols indicate significant differences between the control and *p*-coumaric acid (^) or the control and ferulic acid (#) on a given day ($p < 0.05$).

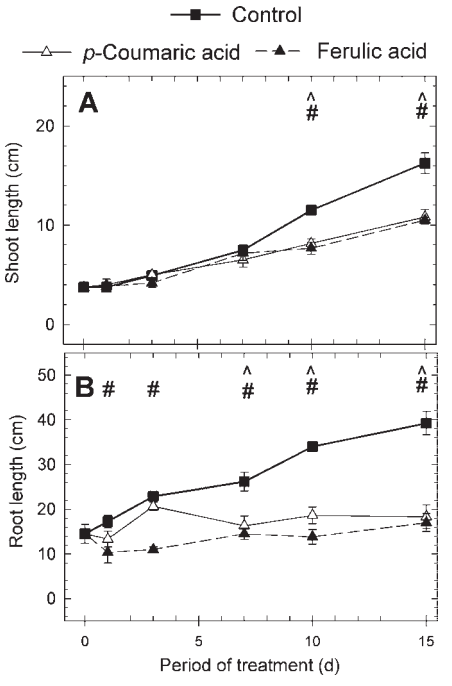

Figure 4. Shoot length (A) and root length (B) of control pea plants or plants treated with *p*-coumaric acid or ferulic acid supplied in the nutrient solution. Each value is the mean ± standard error (n = 8). Symbols indicate significant differences between the control and *p*-coumaric acid (^) or the control and ferulic acid (#) on a given day ($p < 0.05$).

within the first day, and the shoot growth was arrested after 10 days (Figure 4). Benzoic and cinnamic acid derivatives are among the most studied and well-known allelochemicals, probably because of their wide distribution,[20] and the inhibitory effects of ferulic acid and *p*-coumaric acid on the growth of different species have been widely reported.[19,21−24]

To evaluate whether the exogenous supply of ferulic acid or *p*-coumaric acid mimicked the herbicide treatment, the most significant physiological effects detected after ALS inhibition, including the carbohydrate content, ethanolic fermentation, and quinate content, were assessed in the plants supplied with ferulic acid and *p*-coumaric acid. Although the sucrose and starch contents were not affected in the roots of treated plants (data not shown), soluble sugars and starch accumulated in the leaves of pea plants treated with ferulic acid or *p*-coumaric acid (Figure 5). Fructose accumulation was only evident at the initial and final phases of the treatment, whereas sucrose and glucose accumulated throughout the whole treatment, and starch accumulated starting on day 7. Similarly, carbohydrate accumulation has been detected in the leaves of plants treated with ALS inhibitors; after herbicide treatment, the increased carbohydrate content in the leaves is caused by a decrease in sink strength because unmetabolized carbohydrates had accumulated in the roots.[3] Soybean roots supplied with ferulic acid accumulated carbohydrates, but no conclusions regarding the physiological causes of the carbohydrate accumulation were provided.[25] It is possible that respiration might be involved because respiration is the main pathway that consumes carbohydrates, and the effects of phenolic acids on the overall respiratory metabolism are stimulatory or inhibitory depending on the phenolic acid concentration.[20]

After the exogenous supplementation of ferulic acid and *p*-coumaric acid for 7 and 15 days, the induction of PDC and ADH protein expression was detected (Figure 6). Similarly, ALS inhibition increased aerobic fermentation after, due to increases in the levels of fermentative enzymes.[4] Two facts have been proposed that are related to the induction of aerobic fermentation after ALS inhibition. First, pyruvate is a common substrate of ALS and PDC enzymes, and ALS inhibition involves the increased

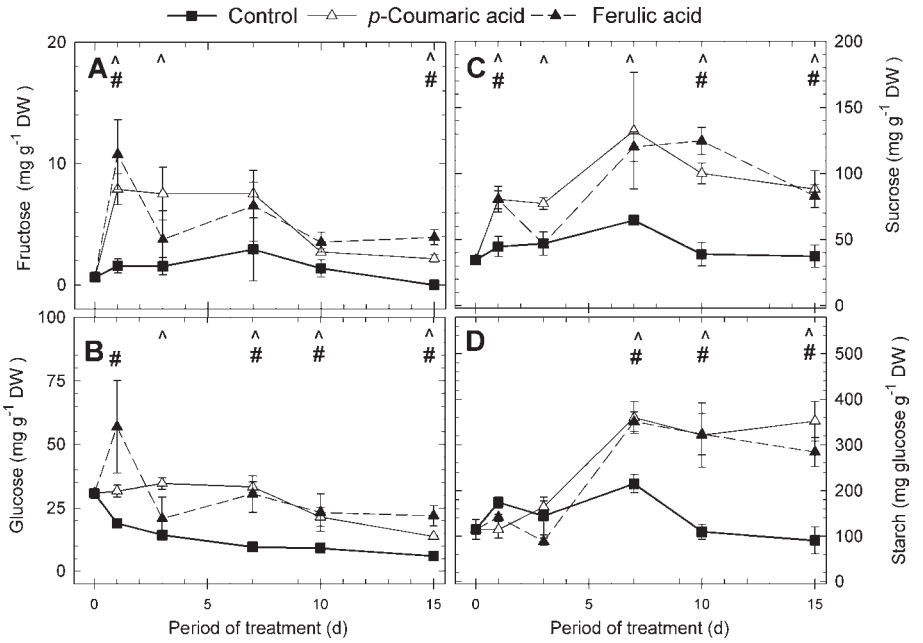

Figure 5. Fructose (A), glucose (B), sucrose (C), and starch (D) content in the leaves of control pea plants or those treated with *p*-coumaric acid or ferulic acid supplied in the nutrient solution. Each value is the mean ± standard error (n = 4). Symbols indicate significant differences between the control and *p*-coumaric acid (^) or the control and ferulic acid (#) on a given day ($p < 0.05$).

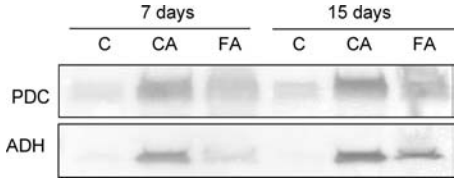

Figure 6. Immunoblots of PDC and ADH in the roots of control (C) pea plants or those treated with *p*-coumaric acid (CA) or ferulic acid (FA) at 7 or 15 days after treatment. Each lane contains 40 μg of protein.

availability of pyruvate for other enzymes, such as PDC.[26] Second, some abiotic stresses that are not related to hypoxia induce the expression of ADH and PDC.[27,28] Indeed, it has been proposed that fermentation has a general function in aerobic metabolism under stress conditions and may be an important switch in regulating carbohydrate metabolism.[29] Fermentative induction after the treatment of hydroxycinnamic acids is not easily correlated to pyruvate availability. Therefore, the fermentative response may be considered a physiological effect that is induced under stress. Ferulic acid and *p*-coumaric acid caused an accumulation of quinate in pea plant leaves from the initial stages of treatment (Figure 7). Quinate was accumulated after ALS inhibition[6] and was a common effect of ALS inhibitors and the herbicide glyphosate. Also, the exogenous application of quinate partially mimicked herbicide treatment, suggesting a role for quinate in the mode of action of ALS inhibitors and glyphosate.[6] In the present study, quinate was also accumulated in leaves after the exogenous application of ferulic acid and *p*-coumaric acid, suggesting cross-effects among the three compounds.

The exogenous application of ferulic acid or *p*-coumaric acid to pea plants caused the internal accumulation of ferulic acid or *p*-coumaric acid, growth arrest, carbohydrate and quinate accumulation in the leaves and the induction of ethanolic fermentation

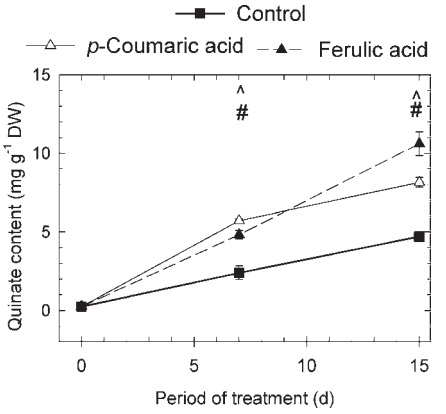

Figure 7. Quinate content in the leaves of control pea plants or plants treated with *p*-coumaric acid or ferulic acid supplied in the nutrient solution. Each value is the mean ± standard error (n = 4). Symbols indicate significant differences between the control and *p*-coumaric acid (^) or the control and ferulic acid (#) on a given day ($p < 0.05$).

(Figures 3−7). All of these results resemble some of the physiological effects that have been detected after ALS inhibitor treatment and suggest the possibility that ferulic acid and *p*-coumaric acid play a role in the mode of action of herbicides that inhibit branched chain amino acid biosynthesis. Cinnamic acid derivatives do not present a sole target in the cell but affect many physiological processes.[20] It is difficult to discern whether some of the specific physiological effects are primary or secondary consequences of the treatment.[11]

The inhibitory effects of ferulic acid and *p*-coumaric acid treatments on plant growth have been related to an increase in the lignin content.[23,24,30] Two explanations have been proposed to link the supply of these phenolic acids and the increased lignification process. First, it has proposed a model of the mode

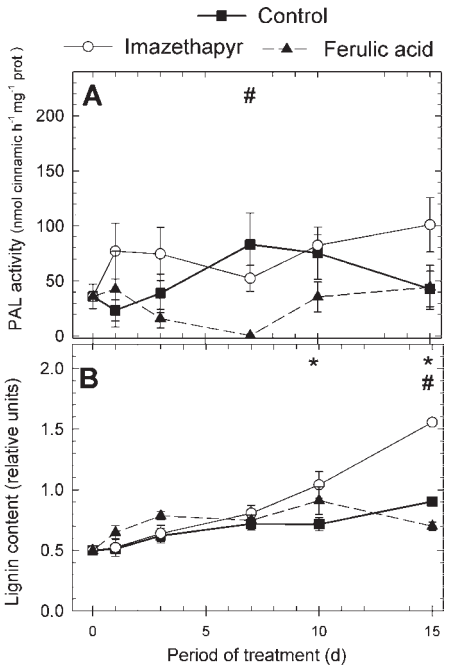

Figure 8. Phenylalanine amino lyase activity (A) and lignin content (B) in the roots of control pea plants or plants treated imazethapyr or ferulic acid supplied in the nutrient solution. Each value is the mean ± standard error (n = 4). Symbols indicate a significant difference between the control and imazethapyr (*) or the control and ferulic acid (#) on a given day ($p < 0.05$).

of action of phenolic acids and suggested that increased lignification is a secondary effect of oxidative stress.[31] In this model,[31] the activity of phenolic acids is associated with their transformation into oxidized forms. During phenol oxidation, reactive oxygen species are produced that are responsible for lipid peroxidation. A consequence of lipid peroxidation may be the induction of lipoxygenase, which may initiate a chain of reactions leading t o ethylene production. Ethylene production is followed by PAL induction, which increases lignin synthesis, contributing to growth inhibition. Second, ferulic acid and *p*-coumaric acid may be directly channeled into the phenylpropanoid pathway and may increase the amount of lignin monomers, solidifying the cell wall and restricting root growth.[24,32] Lignin is synthesized from phenylalanine, which is an end product of the shikimate pathway, and lignin accumulation is accompanied by the activation of enzymes that mediate lignin biosynthesis, such as PAL. Although PAL activity is increased after ALS inhibition by chlorsulfuron in sunflower hypocotyls,[8] measurements of the lignin content in plants treated with ALS inhibitors have not been published previously. Therefore, we investigated the effects of ferulic acid and imazethapyr treatments on both the PAL activity and the lignin content (Figure 8).

Although PAL activity was induced after the supply of the sylfonylurea, chlorsulfuron,[9] no effect on PAL root activity was detected after the supply of the imidazolinone imazethapyr (Figure 8A). PAL activity only showed transient decrease after 7 days of ferulic acid treatment (Figure 8A). PAL activity may be inhibited,[24] stimulated,[30] or unaffected[33] by different phenolic allelochemicals. Although ferulic acid has been previously shown to promote a general increase in lignin content, we only detected a transient, nonsignificant increase in the lignin content in the roots of plants treated with ferulic acid (Figure 8B). This difference might be due to the duration of the experiment, as our study (2 weeks) was much longer than those previously reported (usually no longer than 3 days).

To our knowledge, this is the first report studying effects of ALS inhibitors on lignin content. We found that the lignin content was increased in imazethapyr-treated roots at day 10 from the onset of treatment (Figure 8B). Although assessing the lignification at the end of the study appeared to provide a relation with the root growth, it is not likely to be the only cause because the growth inhibition was detected only 24 h after the onset of the treatment. The lignification was probably due to a greater carbon flow through the shikimate pathway, which was indicated by the showed increased amount of DAHPS (Figure 2).

Currently, new pesticides are being developed to replace the compounds that no longer meet legislative requirements. The discovery of more compounds is needed to combat the evolution of resistance.[34] In this context, natural product-based herbicides are being discovered, and phenolic derivatives may play an important role because of their well-established allelopathic effects. In the current study, the mode of action of ferulic acid and *p*-coumaric acid was evaluated, and we identified several similarities with the mode of action of herbicides that inhibit branched chain amino acid biosynthesis.

AUTHOR INFORMATION

Corresponding Author

*Tel: +34948169120. Fax: +34948168930. E-mail: royuela@unavarra.es.

Funding Sources

This work was supported by the AGL-2007−61134/AGR and the AGL-2010-18621 grants (from the Ministerio de Educación y Ciencia, Spain). L.O. and M.I. received funding from fellowships from the Ministerio de Educación and the Universidad Pública de Navarra, respectively.

ACKNOWLEDGMENT

We thank G. Garijo for technical assistance.

ABBREVIATIONS USED

ADH, alcohol dehydrogenase; ALS, acetolactate synthase; DAHPS, 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase; PAL, phenylalanine ammonia lyase; PDC, pyruvate decarboxylase

REFERENCES

(1) Ray, T. B. The mode of action of chlorsulfuron: A new herbicide for cereals. *Pestic. Biochem. Physiol.* **1982**, *17*, 10–17.

(2) Shaner, D. L.; Anderson, P. C.; Stidham, M. A. Imidazolinones: Potent inhibitors of acetohydroxyacid synthase. *Plant Physiol.* **1984**, *76*, 454–546.

(3) Zabalza, A.; Orcaray, L.; Gaston, S.; Royuela, M. Carbohydrate accumulation in leaves of plants treated with the herbicide chlorsulfuron or imazethapyr is due to a decrease in sink strength. *J. Agric. Food Chem.* **2004**, *52*, 7601–7606.

(4) Zabalza, A.; González, E. M.; Arrese-Igor, C.; Royuela, M. Fermentative metabolism is induced by inhibiting different enzymes of the branched-chain amino acid biosynthesis pathway in pea plants. *J. Agric. Food Chem.* **2005**, *53*, 7486–7493.

(5) Gaston, S.; Ribas-Carbo, M.; Busquets, S.; Berry, J. A.; Zabalza, A.; Royuela, M. Changes in mitochondrial electron partitioning in

response to herbicides inhibiting branched-chain amino acid biosynthesis in soybean. *Plant Physiol.* **2003**, *133*, 1351–1359.

(6) Orcaray, L.; Igal, M.; Marino, D.; Zabalza, A.; Royuela, M. The possible role of quinate in the mode of action of glyphosate and acetolactate synthase inhibitors. *Pest Manage. Sci.* **2010**, *66*, 262–269.

(7) Suttle, J. C.; Schreiner, D. R. Effects of DPX-4189 (2-chloro-N-((4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl)benzene sulfonamide) on anthocyanin synthesis, phenylalanine ammonia lyase activity, and ethylene production in soybean hypocotyls. *Can. J. Bot.* **1982**, *60*, 741–745.

(8) Suttle, J. C.; Swanson, H. R.; Schreiner, D. R. Effect of chlorsulfuron on phenylpropanoid metabolism in sunflower seedlings. *J. Plant Growth Regul.* **1983**, *2*, 137–149.

(9) Nemat-Alla, M. M.; Younis, M. E. Herbicide effects on phenolic metabolism in maize (*Zea mays* L.) and soybean (*Glycine max* L.) seedlings. *J. Exp. Bot.* **1995**, *46*, 1731–1736.

(10) Inderjit; Duke, S. O. Ecophysiological aspects of allelopathy. *Planta* **2003**, *217*, 529–539.

(11) Weir, T. L.; Park, S. W.; Vivanco, J. M. Biochemical and physiological mechanisms mediated by allelochemicals. *Curr. Opin. Plant Biol.* **2004**, *7*, 472–479.

(12) Einhellig, A. F. Mechanisms of action of allelochemicals in allelopathy. In *Allelopathy: Organisms, Processes and Applications*; Inderjit; Dakshimini, K. M. M.; Einhellig, A. F., Eds.; ACS Symposium Series 582; American Chemical Society: Washington, DC, 1995; pp 96−117.

(13) Politycka, B.; Mierlcarz, B. Involvement of ethylene in growth inhibition of cucumber roots by ferulic acid and *p*-coumaric acids. *Allelopathy J.* **2007**, *19*, 451–460.

(14) Zabalza, A.; van Dongen, J. T.; Froelich, A.; Oliver, S. N.; Faix, B.; Gupta, K. J.; Schmälzlin, E.; Igal, M.; Orcaray, L.; Royuela, M.; Geigenberger, P. Regulation of respiration and fermentation to control the plant internal oxygen concentration. *Plant Physiol.* **2009**, *149*, 1087–1098.

(15) Pinto, J. E. B. P.; Dyer, W. E.; Weller, S. C.; Herrmann, K. M. Glyphosate induces 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase in potato (*Solanum tuberosum L.*) cells grown in suspension culture. *Plant Physiol.* **1988**, *87*, 891–893.

(16) Dyer, W. E.; Henstrand, J. M.; Handa, A. K.; Herrmann, K. M. Wounding induces the first enzyme of the shikimate pathway in Solanaceae. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 7370–7373.

(17) McCue, K. F.; Conn, E. E. Induction of 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase activity by fungal elicitor in cultures of *Petroselinum crispum*. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 7374–7377.

(18) Keith, B.; Dong, X. N.; Ausubel, F. M.; Fink, G. R. Differential induction of 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase genes in *Arabidopsis thaliana* by wounding and pathogenic attack. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 8821–8825.

(19) Vaughan, D.; Ord, B. Influence of phenolic acids on morphological changes in roots of *Pisum sativum*. *J. Sci. Food Agric.* **1990**, *52*, 289–299.

(20) Macías, F. A.; Molinillo, J. M. G.; Varela, R. M.; Galindo, J. C. G. Allelopathy—A natural alternative for weed control. *Pest Manage. Sci.* **2007**, *63*, 327–348.

(21) Politycka, B. Phenolics and the activities of phenylalanine ammonia-lyase, phenol-β-glucosyltransferase and β-glucosidase in cucumber roots as affected by phenolic allelochemicals. *Acta Physiol. Plant.* **1998**, *20*, 405–410.

(22) Baleroni, C. S. S.; Ferrarese, M. L. L.; Braccini, A. L.; Scapim, C. A.; Ferrarese-Filho, O. Effects of ferulic and *p*-coumaric acids on canola (*Brassica napus* L. cv. Hyola 401) seed germination. *Seed Sci. Technol.* **2000**, *28*, 333–340.

(23) Dos Santos, W. D.; Ferrarese, M. L. L.; Finger, A.; Teixeira, A. C. N.; Ferrarese-Filho, O. Lignification and related enzymes in *Glycine max* root growth-inhibition by ferulic acid. *J. Chem. Ecol.* **2004**, *30*, 1203–1212.

(24) Zanardo, D. I. L.; Lima, R. B.; Ferrarese, M. L. L.; Bubna, G. A.; Ferrarese-Filho, O. Soybean root growth inhibition and lignification induced by *p*-coumaric acid. *Environ. Exp. Bot.* **2009**, *66*, 25–30.

(25) Ferraresese, M. L. L.; Souza, N. E.; Rodrigues, J. D.; Ferrarese-Filho, O. Carbohydrate and lipid status in soybean roots influenced by ferulic acid uptake. *Acta Physiol. Plant.* **2001**, *23*, 421–427.

(26) Gaston, S.; Zabalza, A.; Gonzalez, E. M.; Arrese-Igor, C.; Aparicio-Tejo, P. M.; Royuela, M. Imazethapyr, an inhibitor of the branched-chain amino acid biosynthesis, induces aerobic fermentation in pea plants. *Physiol. Plant* **2002**, *114*, 524–532.

(27) Dolferus, R.; Jacobs, M.; Peacock, W. J.; Dennis, E. S. Differential interactions of promoter elements in stress responses of the Arabidopsis Adh gene. *Plant Physiol.* **1994**, *105*, 1075–1087.

(28) Kürsteiner, O.; Dupuis, I.; Kuhlemeier, C. The pyruvate decarboxylase1 gene of Arabidopsis is required during anoxia but not other environmental stresses. *Plant Physiol.* **2003**, *132*, 968–978.

(29) Tadege, M.; Dupuis, I.; Kuhlemeier, C. Ethanolic fermentation: New functions for an old pathway. *Trends Plant Sci.* **1999**, *4*, 320–325.

(30) Politycka, B. Ethylene-dependent activity of phenylalanine ammonia-lyase and lignin formation in cucumber roots exposed to phenolic allelochemicals. *Acta Soc. Bot. Pol.* **1999**, *68*, 123–127.

(31) Politycka, B. Physiological responses of cucumber to allelochemicals of phenolic compounds. *Allelopathy J.* **2002**, *10*, 85–104.

(32) Dos Santos, W. D.; Ferrarese, M. L.; Nakamura, C. V.; Mourão, K. S.; Mangolin, C. A.; Ferrarese-Filho, O. Soybean (*Glycine max*) root lignification induced by ferulic acid. The possible mode of action. *J. Chem. Ecol.* **2008**, *34*, 1230–1241.

(33) Shann, R.; Blum, U. The utilization of exogenously applied ferulic acid in lignin biosynthesis. *Phytochemistry* **1987**, *26*, 2977–2982.

(34) Dayan, F. E.; Cantrell, C. L.; Duke, S. O. Natural products in crop protection. *Bioorg. Med. Chem.* **2009**, *17*, 4022–4034.