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Interaction of Gibberellic Acid and Glyphosate on Growth and Phenolic Metabolism in Soybean Seedlings

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Abstract: The plant growth regulator gibberellic acid (GA) and the herbicide glyphosate were examined for their possible interactions with growth and phenolic metabolism in soybean [*Glycine max* (L.) Merr. Cv. Hill] seedlings. GA caused increases in phenylalanine ammonia-lyase activity (PAL) (per axis basis) above those of the control seedling levels 48 h after treatment in light-grown seedlings. This effect increased to two-fold greater than control levels by 72 and 96 h after treatment. In dark-grown plants, GA had no effect on PAL levels at 24 h, reduced levels at 48 and 72 h, and increased PAL at 96 h. Early studies in our lab reported that glyphosate increased PAL levels, and also reduced hydroxyphenolic compound accumulation in both light- and dark-grown soybean seedlings. Treatments of GA plus glyphosate caused additive increases in PAL activity in light-grown seedlings, but GA lowered glyphosate's increase in PAL levels at 48–96 h after treatment in dark-grown seedlings. GA had little effect on hydroxyphenolic compound levels in either light- or dark-grown seedlings. GA treatment alone did not significantly affect root elongation, but stimulated hypocotyl and epicotyl elongation and caused marginal reversal of glyphosate inhibition of elongation in roots, hypocotyls, and epicotyls in light-grown plants. These results show some differential effects of GA and glyphosate on growth and phenolic metabolism, and their interactions that are dependent on plants grown in light or darkness.

Keywords: gibberellic acid; GA; glyphosate; phenolic compounds; PAL; phenylalanine ammonia-lyase

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1. Introduction

Gibberellic acids (GAs), or gibberellins, are naturally occurring compounds with plant growth regulatory activity used to stimulate cellular division and/or elongation in leaf and stem tissues [1]. Discovery of these compounds began during the early 19th century, with observations of the effects of a fungal disease on rice (*Oryza sativa* L.) that caused excessive shoot elongation, with major research occurring on their biosynthesis and effects in higher plants in the late 1950s, as reviewed by Hedden and Sponsel [2]. The causal agent of this rice disease, *Gibberella fujikuroi* (aka, *Fusarium fujikuroi*), is a species complex comprising numerous phylogenetically related species [3,4]. Since the discovery of GA, numerous studies have been published on its interactions in plants. GA has been used as a tool to enhance growth, development, and productivity in many species of plants via its ability to alter dormancy, flowering, and fruiting. Currently gibberellic acid (GA) has many uses in agriculture and is available in an array of formulations [5].

GA affects a vast array of biological, biochemical, and physiological parameters in horticultural, vegetable, agronomic, and weed plants, and it can increase cell division and improve crop yield. For example, GA produced in tomato (*Lycopersicon esculentum* L.) cotyledon was found to be necessary for cell division to occur during reunion in the cortex of cut hypocotyls of tomato and cucumber (*Cucumis sativus* L.) [6]. GA was also shown to reverse male sterility in tomato [7]. Induced anthocyanin synthesis, caused by GA in petunia (*Petunia x hybrida)* tissues, was attributed to increased activity of chalcone synthase

and chalcone flavanone isomerase [8]. The levels of total phenolic compounds, tannins, and anthocyanins have been altered or increased by GA in grapes (*Vitis vinifera* L.) in attempts to improve quality [9]. Studies of the effects of GA on antioxidant activity in germinating soybean seeds showed that this compound enhanced the production of antioxidant compounds including phenolics and flavonoids [10]. GA had remedial effects on pollen viability in glyphosate [N-(phosphonomethyl)glycine]-resistant cotton (*Gossypium hirsutum* L.) plants [11] and on enhancement of cotton boll retention [12]. GA was also shown to stimulate the germination of several vegetable crops [onion (*Allium cepa* L.), beet (*Beta vulgaris* L.), carrot (*Daucus carota* L.)], and the emergence of two major species of weeds [Palmer amaranth (*Amaranthus palmerii*) and velvetleaf (*Abutilon theophrasti* L.)], but the emergence of redroot pigweed (*Amaranthus retroflexus* L.) was not affected [13]. Supplemental indole acetic acid (IAA) application was found to inhibit the seed germination of soybean via a mechanism that increases abscisic acid (ABA) production and lowers GA synthesis, thereby lowering the GA/ABA ratio [14]. Various interactions of GA with several herbicides have also been reported. The herbicide alachlor [2-chloro-2′,6′-diethyl-N-(methoxy-methyl)acetanilide] significantly inhibited corn (*Zea mays* L.) epicotyl growth, whereas GA was stimulatory to the growth of these plant tissues [15]. Interactions occurred when these two compounds were combined, i.e., growth was increased compared to alachlor treatment alone, decreased compared to GA alone, and essentially equal to that of the untreated control tissue. Combinations of the herbicide prometryn (1,3,5-triazine-2,4-diamine) with GA caused increased phytotoxicity to black nightshade (*Solanum nigrum* L.), a weed from the Solanaceae family that contains toxic alkaloids [16]. Studies to examine the interactions of GA with two herbicides (glyphosate and fluazifop {2-[4-[5-(trifluoromethyl)pyridin-2-yl]oxyphenoxy]propanoic acid}) in water-stressed oat (*Oryza sativa* L.) under different nitrogen levels, showed that GA applied prior to herbicide application could result in the higher efficacy of both compounds under a low nitrogen regime [17]. Interaction studies of GA and glyphosate examined sorghum (*Sorghum bicolor* L.) seeds and showed that the herbicide altered seed germination and caused interactions with their oxidative metabolism and GA synthesis [18]. Other studies of GA interactions with glyphosate showed that spray applications of glyphosate decreased sugarcane yield but raised sugar content, while GA application increased both yield and sugar accumulation [19]. When combined, GA plus glyphosate spray applications provided higher yields of sugar and sugarcane than the application of either compound alone. Glyphosate was reported to directly affect components of GA biosynthesis in leafy spurge (*Euphorbia esula* L.) tissue [20]. GA application has been shown to increase glyphosate toxicity on bean plants (*Phaseolus vulgaris* L.) and Canada thistle (*Cirsium arvense* L.) [21]. More recently, a patent was granted describing methods to control Palmer amaranth (*Amaranthus palmeri*), waterhemp (*A. rudis*) and smooth pigweed (*A. quitensis*) using compositions with the herbicide flumioxazin [2-(7-fluoro-3-oxo-4-prop-2-ynyl-1,4-benzoxazin-6-yl)-4,5,6,7-tetrahydroisoindole-1,3-dione] and GA [22]. GA has been reported to hasten maturity, improve yield, and increase herbicide tolerance in wheat [23,24]. More recently, the application of micro-encapsulated GA was found to reduce wheat injury from methyl-sulfuron [methyl 2-{[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)carbamoyl]-sulfamoyl}benzoate] application [25].

The enzyme phenylalanine ammonia-lyase (PAL) is pivotal in the phenylpropanoid metabolic pathway. It catalyzes the transformation of L-phenylalanine to yield *t*-cinnamic acid and ammonia, and leads to the accumulation of other metabolic products (lignins, flavonoids, coumarins, etc.) [26]. Certain plant hormones and growth regulators, such as IAA and 2,4-dichlorophenoxy acetic acid (2,4-D), are recognized to have major effects on the regulation of PAL activity in various plant species [27]. These compounds, regulated by PAL activity, are essential for plant growth and development, e.g., lignins for mechanical strength [28], anthocyanins and others as pigments [29], and stress factors (biotic/abiotic) [30] defend against wounding and pathogen attack [31]. A fungal pathogen, *Fusarium fujikuroi*, can produce high amounts of GA and causes bakanae disease in rice [32].

In a resistant rice cultivar, this fungus was found to induce high phytoalexin production, with no disease symptomology. However, in a susceptible cultivar, disease symptoms were exhibited, GA and ABA levels were elevated, and jasmonic acid and phytoalexin production were inhibited, suggesting that many diverse secondary compounds are active in plant defense and that phytoalexin production may play a defense role against this disease in rice. [33]. Effects of GA on secondary plant metabolism include the suppression of light-induced PAL activity in lettuce (*Lactuca sativa* L.) seedlings [34], and PAL activity increases caused by GA in corn [35], strawberry (*Fragaria vesca*) [36], and pea (*Pisum sativum*) [37].

Due to the widespread continued use and interest in glyphosate, and because GA is used in various agricultural situations and interacts with glyphosate and some other herbicides and alters accumulations of plant enzymes and constituents related to secondary plant metabolism, our objectives were to investigate the interaction of GA and GA combined with glyphosate on plant growth and aspects of secondary plant metabolism (PAL and associated phenylpropanoid plant products). We chose non-GMO soybean [*Glycine max* (L.) Merr. 'Hill'] as a test plant, since our laboratory has previously conducted several studies on the interactions of glyphosate and other herbicides with secondary plant metabolism using this crop plant. For example, the effects of various herbicides from 14 chemical classes on soluble protein, hydroxyphenolic, anthocyanin, and chlorophyll levels were determined using soybean under both light- and dark-growth conditions [38–40], because phenolic metabolism is under photo morphogenic regulation [27]. Furthermore, soybean was used to study interactions of the plant growth regulator IAA and glyphosate related to secondary plant metabolism [41].

2. Materials and Methods

2.1. Plant Material and Growth Conditions

Non-GMO soybean [*Glycine max* (L.) Merr. 'Hill'] seeds were surface-sterilized and germinated in darkness in an environmental growth chamber (Precision Scientific Inc., Chicago, IL, USA) at 25 °C as described [30]. Uniform seedlings were selected after 3 days of growth and transplanted into plastic petri dish lids perforated with holes (each hole containing a seedling) fitted atop petri dishes containing 2 mM $CaSO_4$ (control), $CaSO_4$ with glyphosate (0.5 mM), or with GA (10 μM). In the interaction experiments, glyphosate and GA were prepared together in 2 mM $CaSO_4$ at the concentrations indicated above. Sets of seedlings in dishes were grown under continuous darkness or under continuous light (200 $\mu E \cdot m^{-2} \cdot s^{-1}$, PAR) measured with a light meter (LI-COR, Inc., Lincoln, NE, USA) or at 25 °C. Seedling axes were harvested at various times over a 96 h time course following exposure to these chemical treatments.

2.2. Chemical Sources and Purity

Glyphosate (99.8% pure, free acid) was obtained from Chem Service, Inc. (West Chester, PA, USA). Gibberellic acid (GA_3) was a product from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were high-purity (ACS reagent grade) products.

2.3. PAL Extraction and Assay

PAL in soybean axis tissue was extracted, partially purified, and assayed as before [42]. Axis tissue of 12 to 18 seedlings was used for each preparation. Enzyme activity is given as ηkat, where one unit is equal to the amount of enzyme required to produce 1 μmol of product (*t*-cinnamic acid) from the substrate (L-phenylalanine) min^{-1} at 30 °C (39). The protein in the enzyme preparations was determined using the Bradford reagent [43].

2.4. Total Hydroxyphenolic Compound Content

The extraction of hydroxyphenolic compounds was achieved by homogenizing soybean tissues at several time points during the treatment time course using alcohol:H_2O (75% aqueous, v/v) using a high-speed electric blender (Virtis 45 Homogenizer, Los Angeles,

CA, USA). The blended mixtures were clarified using centifugation (20 min, 20,000× *g*, 0 °C). The supernatants were then quantitatively assayed for total hydroxyphenolics using a colorimetric–spectrophotometric procedure with the Folin–Ciocalteu reagent [44]. Quantiative measurments were made with a UV spectrophotometer (Agilent 8453, UV–Visible Spectroscopy System; Agilent Technologies, Santa Clara, CA, USA).

2.5. Anthocyanin Content

Several soybean hypocotyls (4 to 6) were excised and then homogenized with 20 mL chilled acidic methanol [MeOH:HCl (1%), *v*/*v*] in an electric blender. The homogenized mixtures were centrifuged (20 min, 20,000× *g*, 0 °C) and anthocyanin in the supernatants was spectrophotometrically quantitated as A_{525}–A_{585} per hypocotyl in 10 mL of extraction medium as described previously [45].

2.6. Statistics

The numbers in vertical columns of the tabular data followed by the same letter are not significantly different at the 95% confidence level, as determined by using a paired *t* test [46]. Error bars in the figures represent one standard error of mean values based on three to four replications of each treatment. The experiments were duplicated.

3. Results

3.1. GA and Glyphosate Effects on Growth

Fresh weight accumulation of soybean axes was not significantly affected by GA at 0.5 mM 96 h after treatment in light- or in dark-grown seedlings, but the combination of GA (10 µM) and glyphosate caused increases in fresh weight above the reduction in this parameter caused by glyphosate alone. (Table 1).

Table 1. Fresh weight accumulation (g) of soybean axes after 96 h treatment.

	g per Axis	
Treatment	**Light**	**Dark**
Control	0.64 [c]	0.83 [c]
GA (10^{-5} M)	0.64 [c]	0.84 [c]
Gly. (5×10^{-4} M)	0.39 [a]	0.69 [a]
GA + Gly. ($10^{-5} + 5 \times 10^{-4}$ M)	0.52 [b]	0.75 [b]

The numbers in vertical columns of the tabular data followed by the same letter are not significantly different at the 95% confidence level, as determined by using a paired *t* test [46].

The effects of these treatments on growth (axis elongation) were determined on the axis parts, roots, hypocotyl, and epicotyl (Table 2). GA caused increased elongation of hypocotyls and epicotyls in the light and only the epicotyls in the dark after 96 h treatment compared to the control seedlings. Glyphosate reduced elongation of all plant parts in the light and dark but a combination of GA with glyphosate resulted in significantly higher elongation than in glyphosate alone in all cases except the root in dark-grown plants.

3.2. GA and Glyphosate Effects on Chlorophyll and Anthocyanin Levels

The total chlorophyll content in hypocotyl tissue was reduced about 10% by GA treatment and 50% by the glyphosate treatment compared to control values, 96 h after treatment (Table 3). The combined treatment of GA plus glyphosate resulted in a 30% reduction in chlorophyll content. Anthocyanin content in hypocotyls was not affected by GA treatment after 96 h of treatment, but in the GA plus glyphosate treatment, levels of this pigment were 60% higher than in glyphosate-alone-treated tissue (Table 4).

Table 2. Elongation (mm) of soybean plant organs as effected by various treatments after 96 h.

Treatment	Light		
	Root	**Hypocotyl**	**Epicotyl**
Control	194 ^{c}	68 ^{b}	220 ^{c}
GA (10^{-5} M)	183 ^{c}	110 ^{c}	71 ^{d}
Gly. (5×10^{-4} M)	93 ^{a}	58 ^{a}	2 ^{a}
GA + Gly. ($10^{-5} + 5 \times 10^{-4}$ M)	119 ^{b}	112 ^{c}	7 ^{b}
	Dark		
	Root	**Hypocotyl**	**Epicotyl**
Control	200 ^{c}	188 ^{b}	14 ^{b}
GA (10^{-5} M)	187 ^{b}	193 ^{b}	49 ^{c}
Gly. (5×10^{-4} M)	107 ^{a}	169 ^{a}	5 ^{a}
GA + Gly. ($10^{-5} + 5 \times 10^{-4}$ M)	108 ^{a}	182 ^{b}	8 ^{ab}

The numbers in vertical columns of the tabular data followed by the same letter are not significantly different at the 95% confidence level, as determined by using a paired *t* test [46].

Table 3. Total chlorophyll content in soybean hypocotyls 96 h after chemical and light treatment.

Treatment	Chlorophyll (mg/Hypocotyl)
Control	0.021 ^{c}
GA (10^{-5} M)	0.019 ^{bc}
Gly. (5×10^{-4} M)	0.010 ^{a}
GA + Gly. ($10^{-5} + 5 \times 10^{-4}$ M)	0.014 ^{ab}

The numbers in vertical columns of the tabular data followed by the same letter are not significantly different at the 95% confidence level, as determined by using a paired *t* test [46].

Table 4. Soluble hydroxyphenolic content (µmol) in soybean axes after various chemical treatments.

Treatment	Light			
	24 h	**48 h**	**72 h**	**96 h**
Control	1. 34 ^{a}	2.10 ^{c}	2.35 ^{c}	2.33 ^{b}
GA (10^{-5} M)	1.36 ^{a}	1.90 ^{b}	2.22 ^{b}	2.51 ^{c}
Gly. (5×10^{-4} M)	1.33 ^{a}	1.70 ^{a}	1.79 ^{a}	1.96 ^{a}
GA + Gly. ($10^{-5} + 5 \times 10^{-4}$ M)	1.36 ^{a}	1.92 ^{b}	2.11 ^{b}	2.56 ^{c}
	Dark			
	24 h	**48 h**	**72 h**	**96 h**
Control	1.84 ^{b}	2.14 ^{b}	2.60 ^{c}	2.39 ^{b}
GA (10^{-5} M)	1.81 ^{ab}	2.19 ^{b}	2.44 ^{b}	2.35 ^{b}
Gly. (5×10^{-4} M)	1.77 ^{a}	1.87 ^{a}	2.02 ^{a}	2.12 ^{a}
GA + Gly. ($10^{-5} + 5 \times 10^{-4}$ M)	1.61 ^{a}	2.20 ^{b}	1. 91 ^{a}	2.61 ^{c}

The numbers in vertical columns of the tabular data followed by the same letter are not significantly different at the 95% confidence level, as determined by using a paired *t* test [46].

3.3. GA and Glyphosate Effects on Hydroxyphenolic Compounds and PAL

Under light- and dark-growth conditions, the hydroxyphenolic compound contents were reduced below the control level by glyphosate at most time points of the time course (24–96 h), while GA had little effect (compared to control) in either the dark or light (Table 5). At 72 h, combinations of GA and glyphosate were lower than the control levels in the light or dark, but at 96 h, the combination of glyphosate and GA caused higher contents of these secondary plant products.

Extractable PAL activity in the light-grown soybean axes was increased by GA treatment, but less than the increase caused by glyphosate (Figure 1). The combination of GA plus glyphosate caused exceptionally high PAL levels at 48 to 96 h after treatment. In dark-grown soybean plants, GA caused PAL levels that were below those of control tissue, and the levels of this enzyme were also lower than the treatment of glyphosate alone when GA was supplied with glyphosate (Figure 2).

Table 5. Anthocyanin content * in soybean hypocotyls 96 h after chemical and light treatment.

Treatment	$A_{525–585}$
Control	0.210 [c]
GA (10^{-5} M)	0.210 [c]
Gly. (5×10^{-4} M)	0.102 [a]
GA + Gly. ($10^{-5} + 5 \times 10^{-4}$ M)	0.170 [b]

* $A_{525–585}$ in 10 mL acidic methanol. The numbers in vertical columns of the tabular data followed by the same letter are not significantly different at the 95% confidence level, as determined by using a paired *t* test [46].

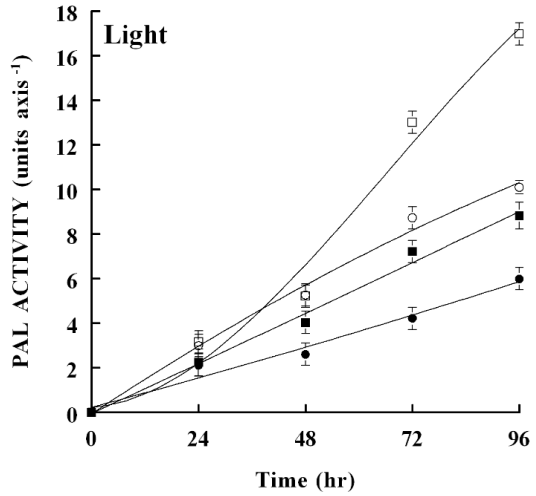

Figure 1. Effects of chemical treatments on extractable PAL activity (units per axis) from 3-day-old dark-grown soybean seedlings, treated and grown in continuous white light over a 96 h time course. Control seedlings (2 mM $CaSO_4$) = closed circles, glyphosate (0.05 mM) = open circles, GA (10 μM) = closed squares, GA (10 μM) + glyphosate (0.50 mM) = open squares. Curve-fit coefficients for PAL-light graphs (all are second-degree polynomials): Control: $Y = 0.232 + 0.054\ X + 0.002\ X^2$; R2 = 0.97; GLY: $Y = -0.062 + 0.133\ X - 0.001\ X^2$; $R^2 = 0.98$; GA: $Y = -0.041 + 0.930\ X + 0.001\ X^2$; $R^2 = 0.98$; GA + GLY: $Y = 0.234 + 0.014\ X + 0.003\ X^2$; $R^2 = 0.98$.

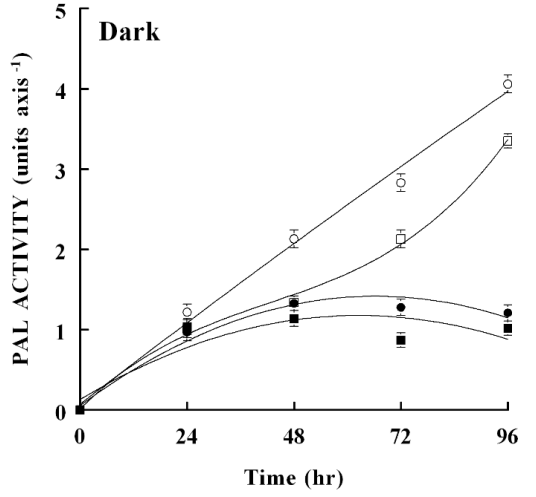

Figure 2. Effects of chemical treatments on extractable PAL activity (units per axis) from 3-day-old dark-grown soybean seedlings, treated and grown in continuous darkness over a 96 h time course. Control seedlings (2 mM $CaSO_4$) = closed circles, glyphosate (0.05 mM) = open circles, GA (10 μM) = closed squares, GA (10 μM) + glyphosate (0.50 mM) = open squares. Curve-fit coefficients for PAL-dark graphs (all are second-degree polynomials): Control: $Y = 0.063 + 0.041\ X - 0.001\ X^2$; R2 = 0.97; GLY: $Y = 0.075 + 0.043\ X - 0.002\ X^2$; $R^2 = 0.98$; GA: $Y = 0.133 + 0.033\ X - 0.001\ X^2$; $R^2 = 0.90$; GA + GLY: $Y = 0.17 + 0.055\ X - 0.001\ X^2$; $R^2 = 0.98$.

4. Discussion

In the present study, growth reductions (stem elongation and fresh weight accumulation) in soybean, caused by glyphosate were partially reversed by the supplemental additions of GA. GA partially reversed glyphosate's effect of lowering the hydroxyphenolic compound content in both light- and dark-grown seedlings over a 96 h time course. The reduction in anthocyanin and chlorophyll in hypocotyl tissues caused by glyphosate was also partially reversed by GA. GA elevated PAL activity in plants grown under these light conditions, but lowered their activity during dark growth. GA plus glyphosate treatments resulted in PAL levels higher than the increased levels caused by glyphosate in the light. However, in dark-grown tissues, PAL levels in the GA plus glyphosate-treated plants were increased, but they did not attain levels as high as those in the solely glyphosate treatments.

For comparison, in studies of glyphosate's interaction with the plant growth regulator IAA [41], when IAA was applied to soybean seedlings combined with glyphosate, elongation was reduced and fresh weight production was lowered to values that were greater than or equal to those of glyphosate treatment alone. There was no indication of a glyphosate caused reversal of growth inhibition during either light or dark growth. On a per axis basis, IAA treatment only slightly reduced hydroxyphenolic compound accumulation in light-grown tissue, with no effect in dark-grown tissue. Treatment of plants with IAA plus glyphosate during light growth yielded a hydroxyphenolic compound level slightly higher than glyphosate treatment alone, and this effect was more pronounced in dark-grown tissues, resulting in levels significantly higher than in untreated control tissue. Furthermore, IAA caused low or no effect on PAL levels extracted from light- or dark-grown soybean seedlings. However, this growth regulator was antagonistic to the increase in PAL activity levels caused by glyphosate treatment of light-grown plants. Another research report indicated that exogenous auxin repressed germination of soybean seeds via altering the GA:ABA ratio [14].

During initial studies on the mode of action of glyphosate, aromatic amino acid supplements were found to partially reverse glyphosate's growth inhibition [47]. Since then, many laboratories have sought to find chemicals that can interact to either reverse herbicidal effects of glyphosate and various herbicides, or alternatively, to increase the efficacy of a given herbicide via additive and/or synergistic action. The present study, coupled with the companion study on IAA-glyphosate interactions in soybean seedlings [41], provides a template for future studies that could provide novel information relative to herbicide behavior in plants and the action of bioherbicides in weeds. We have studied various bioherbicides (mostly phytopathogenic fungi) for the control of major weeds as outlined elsewhere [48,49] and discovered involvement with secondary metabolism (PAL) in several pathogen–weed interaction studies [50–52]. Secondary plant metabolism is critically involved in plant stress and defense mechanisms [30,31].

Various phytohormones [auxins, gibberellins, abscisic acid (ABA), jasmonic acid, cytokinins, etc.] interact in stress and in defense responses using cross-talk or signaling [53]. Many of these plant hormones are also produced by symbiotic and pathogenic fungi, again suggesting their possible involvement in pathogenicity defense reaction mechanisms [54]. For example, various *Fusarium* spp. are reported to produce GA [55], IAA [56], ABA [57], cytokinins [58], and GA content is often increased in plants during interactions with mycorrhizal microbes [59]. A correlation was found between GA production and virulence in various strains of *Gibberella fujikuroi* [60]. GA has been reported as a virulence factor in bacterial [61] and fungal [62,63] phytopathogens. When challenged by a pathogen (*Fusarium graminearum*), GA reduced disease severity and infection, whereas ABA promoted infection [57,64].

Although these present studies show interesting interactions of plant enzymes and constituents, plant species, chemical concentration, light growth vs. dark growth and timing are important parameters to consider. Since some of these chemicals are plant hormones, concentration changes in treatment solutions applied to plants could result in dramatically different results due to possible phasic responses to exposure of increasing

amounts of a chemical, i.e., hormesis. Therefore, studying various concentrations of individual compounds and their combinations should provide useful information.

5. Conclusions

Gibberellins are known to repress or stimulate plant defense responses depending on the plant–pathogen interaction, so generally, gibberellins tend to increase their susceptibility to necrotrophs and resistance to biotrophs [65]. Thus, the effects of plant growth regulators such as GA might alter bioherbicidal efficacy, since this regulator promotes cell division, cell elongation, and also influences key enzymes. Alternatively, the use of compounds that inhibit GA biosynthesis, i.e., ABA might promote bioherbicidal action. Studies on interaction of GA, ABA, and phenolic compounds in the control of the hypocotyl growth of *Amaranthus caudatus* demonstrated complex effects on growth when these growth regulators were applied to plants [66]. Furthermore, an invention for chemical weed management using combinations of herbicide (flumioxazin) and GA was patented for control of Palmer amaranth and related weeds [22].

We have preliminary results indicating that ABA can antagonize the increases in PAL activity levels caused by GA or by glyphosate in light-grown soybean seedlings (unpublished). We have also previously discovered the synergistic effects of some bioherbicides when combined with glyphosate [48,49]. Therefore, studies using combinations of GA, glyphosate, and a bioherbicide would also be interesting, especially since, presently, the knowledge on the mode of action of bioherbicides is woefully lacking. These suggested studies would also be important, since GA is widely used as an agricultural chemical, and additional knowledge of the interactions of plant growth regulators and herbicides would aid in explaining the physiological and biochemical effects in crop plants and weeds. With regard to weed control, the possibility of using GA to induce the emergence of large numbers of weeds at one time for more effective weed control management strategies has been suggested [13]. Another experimental strategy to control herbicide-resistant wild radish (*Raphanus raphanistrum*) in cereal crops suggested using the GA biosynthetic pathway as a target site. This would involve the development of an inhibitor specific for GA production in the weed (rather than the crop), or by developing crop species that are insensitive to GA repression, thus allowing the use of potent compounds with GA inhibitory activity to control the weed [67]. Data from all of these studies may help in the quest to find management solutions for the more than 530 cases of herbicide-resistant weeds encompassing 272 species spread worldwide [68].

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