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Release of Lateral Buds from Apical Dominance by Glyphosate in Soybean and Pea Seedlings

T. T. Lee

London Research Centre, Agriculture Canada, University Sub Post Office, London, Ontario, Canada, N6A 5B7

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Abstract. Application of a sublethal dose of glyphosate (*N*-[phosphonomethyl]glycine) to the seedlings of soybean (*Glycine max* L. Merr. cv. Evans) and pea (*Pisum sativum* L. cv. Alaska) promoted growth of the cotyledonary and other lateral buds. The pattern of the glyphosate-induced lateral bud growth was different from that induced by decapitation. Under the experimental condition, glyphosate did not kill the apical buds. Feeding stem sections of the seedlings with radiolabeled indole-3-acetic acid ([2-^{14}C]IAA) and subsequent analysis of free [2-^{14}C]IAA and metabolite fractions revealed that the glyphosate-treated plants had higher rates of IAA metabolism than the control plants. The treated pea plants metabolized 75% of [2-^{14}C]IAA taken up in the 4-h incubation period compared to 46.5% for the control, an increase of 61%. The increase was small but consistent in soybean seedlings. As a result, the glyphosate-treated plants had less free IAA and ethylene than the control plants. The increase of IAA metabolism induced by glyphosate is likely to change the auxin-cytokinin balance and contribute to the release of lateral buds from apical dominance in these plants.

Apical dominance is a complex phenomenon known to be affected by a number of factors. Hormonal control as the basis of apical dominance was first proposed by Thimann and Skoog (1934), who demonstrated that application of auxin could replace the stem apex in inhibiting the growth of the lateral buds. Later the concept of regulation by a balance between auxin and cytokinin was developed inasmuch as kinetin was found to be antagonistic to auxin inhibition of lateral bud growth (Wickson and Thimann 1958, Sachs and Thimann 1964). In accordance with this concept, increasing the concentration of cytokinin or decreasing the concentration of auxin should promote lateral bud growth. Re-

lease of lateral buds from apical dominance by the application of cytokinin has been confirmed (Panigrahi and Audus 1966, Catalano and Hill 1969, Schaeffer and Sharp 1969, Ali and Fletcher 1970, Pillay and Railton 1983). Spray of 2,3,5-triiodobenzoic acid, an inhibitor of auxin transport, has been reported to stimulate tillering in barley (Leopold 1949) and wheat (Langer et al. 1973), suggesting a reduced auxin concentration in the basal node region, thus favoring tiller bud growth. Assuming this to be true, reduction of the overall concentration of free auxin by promoting conjugation and oxidation of auxin should achieve the same or even better results. However, a growth regulator with such a potential has not been reported until recently.

Glyphosate (*N*-[phosphonomethyl]glycine), a relatively new herbicide, has been found to promote conjugation and oxidation of indole-3-acetic acid (IAA) in tobacco callus (Lee 1982a,b). Consequently, the level of free IAA in the glyphosate-treated tissue was significantly less than that in the control. This finding indicated a potential of glyphosate in plant growth regulation in addition to weed control, since IAA is a natural hormone involved in various aspects of plant growth and development. This practical implication has led to further studies to relate the effect of glyphosate to certain biochemical and physiological responses of plants known to be sensitive to IAA, such as lateral bud growth, ethylene production, and senescence.

Promotion of lateral bud growth following glyphosate application has been reported in quack grass (Caseley 1972), Bermuda grass (Fernandez 1976), sorghum, and wheat (Baur et al. 1977). Such an effect has been attributed to a change in the auxin-cytokinin balance in the basal internodes through an inhibition of the basipetal transport of auxin by glyphosate (Baur 1979a,b). However, the plant species used for the transport study was different from those used for the study of tillering. Furthermore, the recent finding that glyphosate promotes IAA metabolism may offer an alternative explanation for the reported increase in tillering in glyphosate-treated plants. The objective of the present study is to clarify the effect of glyphosate on apical dominance and its association with IAA metabolism in plants.

Materials and Methods

Plant Material

Soybean (*Glycine max* L. Merr. cv. Evans) and pea (*Pisum sativum* L. cv. Alaska) were grown in soil in clay pots in a growth chamber at 27°C under a 12-h photoperiod. The light (270 $\mu E.m^{-2}s^{-1}$) was provided by a mixture of fluorescent and incandescent lamps. Seedlings of uniform vigor and height were selected for the experiments.

Glyphosate Treatment

Twelve-day-old seedlings were sprayed once with an aqueous solution of glyphosate to wet the upper surface of all leaves. The commercial formulation

Roundup and analytical grade glyphosate (99.9%) (Monsanto) were used with similar results. The effective concentration of glyphosate sprayed varied from 0.2 to 1 mM as the sensitivity of the plants to glyphosate appeared to change from experiment to experiment. Each treatment had 20–30 plants and each experiment was repeated at least twice. Plants from duplicate sets of control were decapitated manually for a comparison of lateral bud growth with that of the glyphosate-treated plants.

Measurement of Lateral Bud Growth

Fifteen days after treatment, the number of the lateral buds developed from each node was counted and their lengths were measured.

Measurement of IAA Metabolism

The effect of glyphosate on IAA metabolism was measured by the method described previously (Lee 1982a). Briefly, 10 days after the glyphosate treatment, 5 g of 1-cm segments of the internodes of the control and the treated seedlings were incubated with [2-^{14}C]IAA (42.2 μCi/μmol; New England Nuclear, Boston) at 25°C for 4 h in a shaking water bath. After the incubation, the tissue was boiled and homogenized with 80% methanol containing 0.02% sodium diethyldithiocarbamate. The residue was washed thoroughly with fresh extraction medium. Methanol was removed *in vacuo* and the remaining aqueous phase was successively extracted with freshly purified diethyl ether at pH 3.5 and 8, and then with 1-butanol at pH 2.5. The methanol-insoluble residue was further extracted with 1 N NaOH for 16 h. Radioactivity was measured by liquid scintillation counting in a Beckman LS-9000 system. Radioactivity recovered in various fractions other than free IAA served as indications of conjugation and oxidation of [2-^{14}C]IAA.

The acidic ether fraction that contained free [2-^{14}C]IAA was freeze-dried and taken up in 0.1 ml HPLC grade methanol. A portion was applied to a programmed HPLC system (Waters) equipped with a μBondpak C_{18} reverse-phase column. The mobile phase was 69% water, 30% MeOH, and 1% acetic acid at pH 3. The free [2-^{14}C]IAA isolated was confirmed by using an anion exchange column, Partisil-10-SAX (Whatman). The conditions for the separation were the same as reported (Lee 1982a, Lee and Dumas 1983) except that a fluorescence detector (Waters) equipped with a 280-nm excitation filter and a 338-nm emision filter was used. The recovery of IAA was estimated by using a separate sample with a known amount of [2-^{14}C]IAA at the beginning of extraction and by counting the radioactivity of [2-^{14}C]IAA recovered at the end of isolation.

Measurement of Ethylene

One to two grams of 1-cm segments freshly cut from the internodes of the seedlings were used for measurement of ethylene production by the reported procedure (Lee and Dumas 1983).

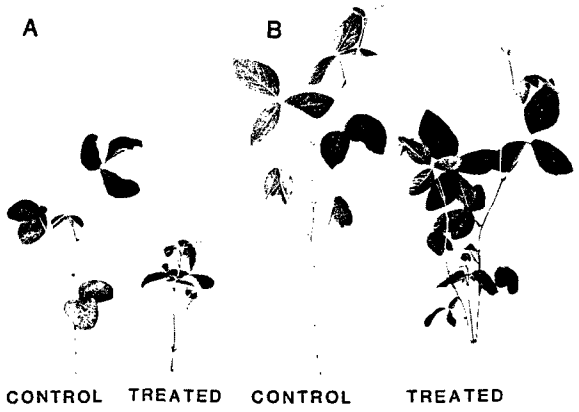

Fig. 1. Promotion of lateral bud growth by glyphosate in soybean seedlings. The concentration of glyphosate was 0.5 mM. A, 15 days after treatment; B, 30 days after treatment.

Fig. 2. Promotion of lateral bud growth by glyphosate in pea seedlings, showing multiple lateral shoots developed 20 days after the spray with 0.5 mM glyphosate.

Results and Discussion

Promotion of Lateral Bud Growth

Application of a sublethal dose of glyphosate to the seedlings of soybean and pea promoted growth of the lateral buds (Figs. 1, 2). Under the experimental condition, glyphosate did not kill the apical buds. An inhibition of growth by glyphosate without killing the tissue has been previously observed in callus cultures of tobacco, soybean, and birdsfoot trefoil (*Lotus corculatus* L.), in which healthy regrowth occurred after the glyphosate-treated calluses were transferred onto a growth medium without glyphosate (Lee 1980b, and unpublished results). Thus, it is evident that the promotion of lateral bud growth by

Table 1. Promotion of lateral bud growth by glyphosate in soybean and pea seedlings.

Plant	Node No.[a]	No. of shoots per node	Length of shoots (cm)
Soybean	Cotyledonary	1.6 ± 0.2	9.6 ± 1.5
Pea	1	1.8 ± 0.1	1.5 ± 0.2
	2	3.0 ± 0	4.1 ± 0.9
	3	2.0 ± 0.1	2.5 ± 0.3
	4	1.5 ± 0.2	2.6 ± 0.3
	5	1.4 ± 0.1	1.8 ± 0.1
	6	1.6 ± 0.1	1.5 ± 0.6

Measurements were taken 15 days after the spray with 0.5 mM glyphosate. The control plants of both species without glyphosate treatment showed no lateral bud growth. Data are the averages of 30 plants ± S.E.

[a] Counted from the base of the stem.

glyphosate in soybean and pea seedlings was not due to death of the apical buds. A similar observation has been reported in sorghum after glyphosate treatment (Baur et al. 1977). The effective concentration of glyphosate appeared to vary with physiological conditions of plants as the lateral shoots of the treated plants grew normally as the main shoots of the control plants (Fig. 1) or, as in other cases, grew little. The time required for promoting lateral bud development by glyphosate was about 5 days.

The pattern of lateral bud growth in the glyphosate-treated soybean and pea seedlings differed from that in the decapitated seedlings. The glyphosate treatment promoted the growth of buds from the cotyledonary and other nodes whereas decapitation usually promoted the growth of the topmost lateral buds. This difference suggests that the release of lateral buds from apical dominance by glyphosate is not equivalent to that caused by the physical removal of the terminal bud.

In the glyphosate-treated soybean seedlings, generally one or both cotyledonary buds of the same plant developed into single shoots (Fig. 1). In the case that both cotyledonary buds developed, one usually grew faster than the other at the beginning and both continued to grow to maturity. Consistent with a preliminary observation, the fresh weight of soybean seeds harvested from the treated plants was as high as that of the control plants. Other lateral buds in addition to the cotyledonary buds also grew when older soybean seedlings were used for the experiments. In the glyphosate-treated pea seedlings, the growth of lateral buds started at the basal node and extended to others in higher positions on the stem with frequent development of multiple shoots from each node (Fig. 2, Table 1). Evidently, the promotion of lateral bud growth by glyphosate is neither limited to the monocotyledonary plants nor restricted to the basal node.

Promotion of IAA Metabolism

Glyphosate treatment increased the rate of IAA metabolism from 46.5% to 75% in pea seedlings as measured at the end of the 4-h period, the increase

Table 2. Effect of glyphosate treatment on [2-^{14}C]IAA metabolism in the internodes of soybean and pea seedlings.

	Total uptake of [2-^{14}C]IAA (dpm · 10^3/g fresh wt)		Free [2-^{14}C]IAA in tissue (dpm · 10^3/g fresh wt)		[2-^{14}C]IAA metabolized (% of total)	
Plant	Control	Treated	Control	Treated	Control	Treated
Soybean	16.5 ± 2.1	17.8 ± 2.9	3.0 ± 0.6	2.6 ± 0.5	81.8	85.4
Pea	11.4 ± 2.3	10.8 ± 2.6	6.1 ± 1.1	2.7 ± 0.4	46.5	75.0

Incubation with [2-^{14}C]IAA was conducted 10 days after application of 0.5 mM glyphosate, and the duration of the incubation was 4 h. Data are the averages of seven experiments for soybean and three experiments for pea.

Table 3. Effect of glyphosate treatment on conjugation of [2-^{14}C]IAA and incorporation of radioactivity into the macromolecular fraction of the internodes of soybean and pea seedlings.

	Radioactivity in BuOH-soluble fraction (% of total uptake)		Radioactivity in MeOH-insoluble fraction (% of total uptake)	
Plant	Control	Treated	Control	Treated
Soybean	58.8 ± 5.2	61.1 ± 7.0	7.1 ± 0.8	14.6 ± 1.8
Pea	25.7 ± 1.2	36.9 ± 2.5	16.2 ± 0.6	19.6 ± 1.0

Incubation with [2-^{14}C]IAA was conducted 10 days after application of 0.5 mM glyphosate, and the duration of the incubation was 4 h. Data are the averages of seven experiments for soybean and three experiments for pea.

was 61% (Table 2). The effect of glyphosate on IAA metabolism was less prominent in soybean than pea. It is interesting to note that the natural rate of IAA metabolism was higher in soybean (81.8%) than pea (46.5%). In a separate study with soybean callus, it was found that the rate of IAA metabolism remained high throughout the growth period. This contrasted with the finding from other tissues, such as tobacco callus, in which the rate of conjugation and oxidation of IAA decreased progressively with increasing growth. These observations suggest that soybean differed from pea or tobacco either in the rate of turnover of free IAA or in the requirement of IAA for growth.

The difference in IAA metabolism between the control and the treated and between soybean and pea seedlings was further demonstrated by the recovery of radioactivity from [2-^{14}C]IAA in two other fractions—the butanol fraction, which contained conjugates of IAA, and the methanol-insoluble fraction, which contained macromolecular constituents of plants (Table 3). In the latter fraction, a major portion of the radioactivity was derived from breakdown products of [2-^{14}C]IAA because less than 10% of the radioactivity in this fraction after alkaline hydrolysis was identified as free [2-^{14}C]IAA. The glyphosate treatment increased the radioactivity in both fractions, indicating increased conjugation and oxidation of IAA. The promotion of IAA metabolism was confirmed by the decrease of free IAA level and ethylene production in the treated plants (Table 4). Glyphosate treatment has been known to decrease ethylene produc-

Table 4. Effect of glyphosate treatment on free IAA level and ethylene production in the internodes of soybean and pea seedlings.

	Free IAA (ng/g fresh wt)		Ethylene (ng/g fresh wt · h)	
Plant	Control	Treated	Control	Treated
Soybean	20.2 ± 1.0	18.9 ± 1.2	1.33 ± 0.04	1.10 ± 0.04
Pea	42.7 ± 1.8	22.1 ± 2.0	1.62 ± 0.14	0.76 ± 0.29

Samples were analyzed 10 days after treatment with 0.5 mM glyphosate.

tion in tobacco callus, and the decrease has been attributed to increased IAA metabolism (Lee and Dumas 1983). In soybean and pea seedlings, the decrease of ethylene production by glyphosate was detectable before the onset of lateral bud development.

The increase of IAA metabolism induced by the glyphosate treatment is likely to alter the auxin-cytokinin balance and to contribute to the release of lateral buds from apical dominance in these plants.

The promotion of IAA metabolism by glyphosate may explain other glyphosate-induced changes, such as the inhibition of growth and the increase of abscission and senescence. These phenomena are known to be regulated by auxin. The reported inhibition of Ca^{2+} uptake by glyphosate (Duke et al. 1983) might also be related to IAA levels in the treated plants, as IAA has been demonstrated to promote the ATP-dependent Ca^{2+} transport in membrane preparations of soybean hypocotyls (Kubowicz et al. 1982).

The target site of glyphosate action has been identified as the 5-enolpyruvylshikimate-3-phosphate synthase (E.C. 2.5.1.19) in the shikimate pathway (Amrhein et al. 1980, Comai et al. 1983, Rogers et al. 1983). Much of the research was done with bacteria. If the inhibition is the primary mode of glyphosate action in plants, links must be found between the inhibition of this specific enzymic step and the various observed glyphosate-induced changes in plants, such as the stimulated lateral bud growth, increased chlorophyll degradation (Abu-Irmaileh and Jordan 1978, Lee 1981), increased ripening in sugarcane (Nickell 1982), decreased ethylene production (Lee and Dumas 1983), inhibited chlorophyll accumulation (Höllander and Amrhein 1980, Kitchen and Witt 1981, Lee 1981), and inhibited ion uptake (Brecke and Duke 1980, Duke et al. 1983). Inhibition of protein synthesis as a result of decrease in the concentration of aromatic amino acids is unlikely to be the answer because the lateral shoots of the glyphosate-treated plants grew normally as the control plants (Fig. 1), and, in certain cases, addition of aromatic amino acids could not reverse the glyphosate-induced symptoms (Brecke 1976, Duke and Hoagland 1978, Cole et al. 1979, Lee 1980b, 1981).

In the shikimate pathway various primary and secondary aromatic metabolites are formed, ranging from aromatic amino acids to phenols and trace factors. Thus, the effect of blocking one early step in the pathway could be far-reaching. As phenols have been shown to affect conjugation and oxidation of IAA in maize stems (Lee 1980a), it is speculated that the reported change in phenolic levels by glyphosate (Hoagland et al. 1978, Holländer and Amrhein

1980, Berlin and Witte 1981, Lee 1982b) may be related to the promotion of IAA metabolism. Thus, the induced change in IAA metabolism may provide a link between the primary mode of action and the induced release of lateral buds from apical dominance as well as certain other IAA-related responses.

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