

## Growth Stimulation of *Euphorbia lathyris* L. by GA<sub>4+7</sub> and BA

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**Abstract.** Container-grown *Euphorbia lathyris* plants were treated with foliar sprays of various combinations of BA and GA<sub>4+7</sub> or 0–3600 mg L<sup>-1</sup> Promalin (1:1 BA + GA<sub>4+7</sub>) in separate experiments. GA<sub>4+7</sub> and Promalin stimulated plants to grow taller. BA and Promalin promoted axillary shoot growth. Multiple applications of Promalin stimulated branching more than single treatments. Dry weight accumulation was stimulated only if the growth regulators were applied to 28–33-cm and not to 56-cm tall plants. Chemical names used: (1 $\alpha$ , 2 $\beta$ , 4 $\alpha$ , 4 $\beta$ , 10 $\beta$ )-2,4a,7-trihydroxy-1-methyl-8-methylenegibb-3-ene-1,10-dicarboxylic acid 1,4a-lactone (GA<sub>4+7</sub>), *N*-(phenylmethyl)-*H*-purin-6-amine (BA), and Promalin [1:1 (wt/wt) GA<sub>4+7</sub> and BA].

A problem with *E. lathyris* is its slow growth rate. In field trials in Illinois, plants grew to only 1-m tall after 1.5 growing seasons (unpublished observations). Researchers reported that a typical crop requires a 7-month growing season to produce plants over 1-m tall (Calvin 1980, Sachs et al. 1981). Sachs et al. (1981) stated that weed control was a problem because of slow canopy closure and no suitable herbicide formulations are known for this crop.

If *E. lathyris* were responsive to growth-promoting plant growth regulators, biomass yield, plant growth, and canopy closure might be increased and rotation time reduced. This study was conducted to determine if the growth and dry matter yield of *E. lathyris* would be promoted by BA, GA<sub>4+7</sub>, or Promalin, the formulated combination of these growth substances.

During the 1980s, *Euphorbia lathyris* L. received considerable attention as a potential bioenergy crop. When comparing the potential of 508 species as renewable energy resources, Roth et al. (1982) rated *E. lathyris* as high as many of the milkweeds (*Asclepias* spp.), and higher than most woody biomass species, including maples (*Acer* spp.), sweet gum (*Liquidambar styraciflua* L.), black locust (*Robinia pseudo-acacia* L.), sycamore (*Plantanus occidentalis* L.), poplars and cottonwood (*Populus* spp.), and willows (*Salix* spp.). Heptane extracts of *E. lathyris* have yielded polycyclic triterpenoids and sterols primarily from the latex (Nemethy et al. 1979). The catalytic cracking of a hexane solvent extract gives a pattern similar to crude oil (Calvin 1980). The remaining dry plant residue can also be used as a biofuel.

The use of the name Promalin or other trade names does not imply endorsement to the exclusion of other products or vendors that may also be suitable.

### Materials and Methods

Seeds were collected from field-grown plants propagated from seeds of a northern California source provided by M. Calvin. Seeds were sown in 15-cm (1800 ml) plastic pots in 2 sphagnum moss peat:1 perlite:1 vermiculite (vol/vol/vol) medium (Promix BX). Plants were grown in the greenhouse at 25/20  $\pm$  5°C (day/night) and received 400 ppm N weekly from a water soluble 20N-8.9P-16.6K fertilizer. Plants were 6–8 weeks old (unless otherwise noted) when first sprayed (75–100 ml/plant) and were sprayed until runoff occurred. The four sprays were water, crystalline GA<sub>4+7</sub> in 50% ethanol and water (vol/vol), the proprietary formulation of BA (ABG-3062), or Promalin (from the commercially available formulation of Abbott Laboratories, North Chicago, IL, USA). Tween 20 was used as a wetting agent at 1 ml L<sup>-1</sup>. When used, multiple applications were at weekly intervals. Dry weight was determined for the above-ground portions (harvestable yield) after drying for 2 days in a drying oven at 60°C. All experiments had at least five replications and were conducted at least twice. Data were pooled across runs for statistical analysis. Main effects and interactions were analyzed when factorial combinations of treatments were used. The  $\sqrt{y} + \frac{1}{2}$  transformation was used when there were many zero values (Steele and Torrie 1980). All data were analyzed using the GLM procedure (SAS Institute, Inc., Cary, NC, USA).

**Table 1.** The effects of BA and GA<sub>4+7</sub> on lateral shoot length (excluding cotyledonary buds) and height of greenhouse-grown *Euphorbia lathyris*.

Plant growth regulator (mg L <sup>-1</sup> )		Plant height <sup>a</sup> (cm)	Number of nodes	Total length of lateral shoots <sup>b</sup> (cm)	
BA	GA <sub>4+7</sub>			Transformed (√y + ½)	Nontransformed
0	0	46.1	36.2	1.9	5.9
0	3	47.0	36.1	2.1	5.8
0	30	51.8 <sup>bc</sup>	37.7*	0.9	0.7
0	300	69.2 <sup>**</sup>	37.2	0.7	0.0
3	0	47.0	36.9	2.8	11.3
3	3	50.2	37.1	1.4	2.3
3	30	51.3*	36.9	0.8	0.1
3	300	81.2 <sup>**</sup>	38.1 <sup>**</sup>	1.0	1.5
30	0	47.9	36.4	2.2	7.6
30	3	50.1	36.9	3.7 <sup>**</sup>	20.1
30	30	51.3*	37.8*	2.0	6.8
30	300	80.4 <sup>**</sup>	37.1	1.4	2.7
300	0	46.7	35.3	3.9 <sup>**</sup>	18.0
300	3	49.1	36.1	3.5 <sup>**</sup>	17.2
300	30	50.9	35.0	2.8	9.3
300	300	78.0 <sup>**</sup>	37.9*	1.4	2.7
Significance <sup>d</sup>		*	*	*	
5% LSD		4.88	1.29	1.10	
1% LSD		6.46	1.71	1.44	

<sup>a</sup> Mean height at treatment time was 28.3 ± 0.4 cm (SE), data were collected 6 weeks later. Each datum point is based on 14 single-plant replications.

<sup>b</sup> Does not include cotyledonary shoots.

<sup>c</sup>\*\*\*Significantly different from the 0 BA, 0 GA<sub>4+7</sub> control at the 5% (\*) or 1% (\*\*\*) level according to *t* test.

<sup>d</sup>\* Significant interaction at the 5% level according to F test with 9 and 195 df.

## Results and Discussion

With a 4 × 4 factorial combination of concentrations of BA and GA<sub>4+7</sub>, there was a significant interaction for plant height, node number, and total length of lateral shoots (excluding cotyledonary shoots; Table 1). Cotyledonary shoots were handled separately because they were much longer than other lateral shoots. Regardless of the level of BA, plants treated with 300 mg L<sup>-1</sup> GA<sub>4+7</sub> were significantly taller than controls (Table 1). Except at 300 mg L<sup>-1</sup> BA, the addition of 30 mg L<sup>-1</sup> GA<sub>4+7</sub> stimulated plants to grow taller than the control plants. Generally, the tallest plants also had the most nodes. The highest levels of BA combined with either 0 or 3 mg L<sup>-1</sup> GA<sub>4+7</sub> stimulated lateral shoot growth compared to control. Enhanced branching occurred only with an exogenous application of cytokinin, whereas high levels of gibberellin were inhibitory. This is unlike the results on

**Table 2.** The effects of GA<sub>4+7</sub> on the growth of greenhouse-grown *Euphorbia lathyris*.<sup>a</sup>

GA <sub>4+7</sub> concentration (mg L <sup>-1</sup> )	Lateral shoots		Cotyledonary bud total length per plant (cm)	Dry wt (g)
	Number ≥ 1 cm			
	Trans-formed (√y + ½)	Nontrans-formed		
0	1.4	2.0	26.0	8.1
3	1.4	2.1	25.9	8.0
30	1.1 <sup>***b</sup>	1.0	27.4	8.5
300	0.9 <sup>**</sup>	0.6	38.7 <sup>**</sup>	10.3 <sup>**</sup>
Significance <sup>c</sup>	**		**	**
5% LSD	0.21		6.17	0.49
1% LSD	0.27		8.18	0.64
Contrast				
Linear	**		**	**
Quadratic	NS		*	**

<sup>a</sup> Main effect data averaged across BA treatments. Each datum point represents the mean of 56 plants.

<sup>b</sup>\*\*\* Significantly different from the 0 GA<sub>4+7</sub> control at the 1% level according to *t* test.

<sup>c</sup>\*\*\* Significant main effect at the 1% level according to F test with 3 and 195 df. NS, not significant.

**Table 3.** The effects of Promalin on greenhouse-grown *Euphorbia lathyris*.<sup>a</sup>

Promalin concentration (mg L <sup>-1</sup> )	Plant height <sup>b</sup> (cm)	No. of nodes	Dry weight (g)
0	40.9	27.8	5.7
6	42.5 <sup>bc</sup>	29.3*	6.3
12	41.9	28.3	6.5
60	44.9 <sup>**</sup>	27.4	6.2
120	46.1 <sup>**</sup>	26.7	6.2
600	58.6 <sup>**</sup>	27.5	7.7 <sup>**</sup>
1200	63.2 <sup>**</sup>	28.3	7.4 <sup>**</sup>
Significance <sup>d</sup>	**	*	**
5% LSD	1.45	1.44	1.00
1% LSD	3.83	1.73	1.18
Contrast			
Linear	**	NS	**
Quadratic	**	NS	NS

<sup>a</sup> Each datum point represents the mean of 10 plants.

<sup>b</sup> Mean height at treatment time was 32.8 ± 0.8 cm (SE).

<sup>c</sup>\*\*\*Significantly different from the 0 Promalin control at the 5% (\*) or 1% (\*\*\*) level according to *t* test.

<sup>d</sup>\*\*\*Significant at the 5% (\*) or 1% (\*\*\*) level according to F test with 6 and 54 df. NS, not significant.

decapitated tomato, where Catalano and Hill (1969) reported a synergistic stimulation of bud outgrowth when kinetin and GA<sub>3</sub> were applied together in lanolin.

As the concentration of BA increased from 0–300

**Table 4.** The effects of multiple applications of Promalin on the growth of greenhouse-grown *Euphorbia lathyris*.

Concentration			Total number			
No. of applications <sup>a</sup>	mg L <sup>-1</sup>		Plant height <sup>b</sup> (cm)	No. of nodes	No. of lateral shoots (≥1 cm)	Dry weight (g)
	Each spray	Total applications				
1	0	0	80.0	63.8	15.3	38.3
	150	150	85.4	62.0	26.9	42.1
	300	300	88.4	64.3	34.0**	41.2
	600	600	96.5***	65.5	23.9	43.1
	900	900	95.5**	63.3	23.8	39.9
	1200	1200	100.8**	63.9	30.8**	44.1
2	0	0	82.0	63.9	21.4	34.0
	150	300	90.8	63.4	36.3**	42.3
	300	600	93.5*	64.3	29.9	38.6
	600	1200	106.5**	63.3	39.6**	43.2
	900	1800	105.2**	62.7	43.8**	42.7
	1200	2400	105.3**	63.9	35.8**	41.1
3	0	0	78.6	65.3	20.0	38.8
	150	450	96.9**	64.3	43.1**	42.4
	300	900	104.5**	63.2	43.2**	40.6
	600	1800	105.3**	62.3	43.6**	39.3
	900	2700	104.7**	63.4	37.8**	40.3
	1200	3600	95.8**	60.9	31.8*	34.4
Significance <sup>d</sup>			**	NS	**	NS
5% LSD			9.21		10.36	
1% LSD			12.15		13.67	

<sup>a</sup> Each datum point represents the mean of 12 plants. Plants were sprayed at weekly intervals.

<sup>b</sup> Mean height at the beginning of the experiment was 55.78 ± 0.8 cm (SE). Data were collected 6 weeks after the last spray.

<sup>c</sup>\*\*\*Significantly different from the 0 control within each number of applications at the 5%(\*\*) or 1%(\*\*\*) level according to the *t* test.

<sup>d</sup>\*\*\*Significant at the 5%(\*\*) or 1%(\*\*\*) level or nonsignificant (NS) according to F test with 17 and 187 df.

mg L<sup>-1</sup>, the number of lateral shoots ≥ 1 cm increased linearly from 0.6–2.5 (data not shown). Imamura and Higaki (1988) also reported a linear increase in number of shoots with increasing BA concentration on *Anthurium* 'Mauna Kea'. Norton and Norton (1986) similarly reported increased shoot number on *Spiraea* and *Arctostaphylos* with increasing BA concentrations of up to 500 mg L<sup>-1</sup>.

As the concentration of GA<sub>4+7</sub> increased, the number of lateral shoots ≥ 1 cm decreased linearly, whereas total length of cotyledonary shoots and plant dry weight increased quadratically (Table 2). The cotyledonary shoots were the most vigorous lateral shoots on the plants and therefore were under the least control by apical dominance. Their stimulation by gibberellin application is similar to the response of buds of other species released from apical dominance by decapitation then stimulated by GA (Catalano and Hill 1969, Imamura and Higaki 1988).

When plants were treated with the formulated mixture of BA and GA<sub>4+7</sub>, Promalin, height increased with increasing concentration in a quadratic manner, the tallest plants were those treated with

1200 mg L<sup>-1</sup> (Table 3). Dry weight increased linearly with increasing Promalin (Table 3). Parameters, such as lateral shoot number and length, were not significantly affected by Promalin (data not shown). Plants receiving the highest dosages were tallest and had the greatest dry weights. These plants appeared most like those that had received the highest dosages of GA<sub>4+7</sub>, described in Tables 1 and 2.

When plants received a total of 600 mg L<sup>-1</sup> Promalin, regardless of multiple or single application, they grew significantly taller than controls (Table 4). There was little advantage from multiple applications. The tallest plants appeared similar to those treated with high levels of GA<sub>4+7</sub> (Tables 1 and 2). These were larger plants and more branched than in previous experiments (Fig. 1; Table 4). The greatest number of branches ≥ 1 cm was on plants receiving ≥ 300 mg L<sup>-1</sup> Promalin in either single or multiple applications. Multiple applications stimulated bud outgrowth more than single applications. Single and double applications of Promalin also caused increased lateral branching compared to controls on pear, cherry, and apple trees (Cody et al. 1985).



Fig. 1. Plants treated with 0 (left) or 150 mg L<sup>-1</sup> Promalin, once, twice, or three times (left to right, respectively).

Unlike applications to smaller (younger) plants, Promalin treatment of larger plants did not increase biomass accumulation as measured by dry weight. Promalin also did not increase apple shoot dry weight when applied as a dip (Young 1987).

This study demonstrates that the growth of *E. lathyris* can be stimulated with applications of gibberellins and cytokinin. To determine if crop rotation time can be shortened to less than 7 months, timing of multiple and single applications should now be studied on field-grown plants under both irrigated and nonirrigated conditions.

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