

# Role of Salicylic Acid and Benzoic Acid in Flowering of a Photoperiod-Insensitive Strain, *Lemna paucicostata* LP6<sup>1</sup>

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## ABSTRACT

*Lemna paucicostata* LP6 does not normally flower when grown on basal Bonner-Devirian medium, but substantial flowering is obtained when 10  $\mu\text{M}$  salicylic acid (SA) or benzoic acid is added to the medium. Benzoic acid is somewhat more effective than SA, and the threshold level of both SA and benzoic acid required for flower initiation is reduced as the pH of the medium is lowered to 4.0. SA- or benzoic acid-induced flowering is enhanced in the simultaneous presence of 6-benzylaminopurine (BAP), although BAP per se does not influence flowering in strain LP6. Continuous presence of SA or benzoic acid in the culture medium is essential to obtain maximal flowering. A short-term treatment of the plants (for first 24 h) with 10  $\mu\text{M}$  SA or benzoic acid, followed by culture in the basal medium containing 1  $\mu\text{M}$  BAP can, however, stimulate profuse flowering. Benzoic acid is more effective than SA, and the effect is more pronounced at pH 4 than at 5.5. Thus, under these conditions, flowering is of an inductive nature. Experiments with [<sup>14</sup>C]SA and [<sup>14</sup>C]benzoic acid have provided evidence that at pH 4 there is relatively more uptake of benzoic acid than SA, thus leading to an increased flowering response. The data obtained from the experiments designed to study the mobility of [<sup>14</sup>C]SA and [<sup>14</sup>C]benzoic acid from mother to daughter fronds indicate that there is virtually no mobility of SA or benzoic acid between fronds.

SA<sup>4</sup> and benzoic acid are known to stimulate flowering in various members of the family Lemnaceae, including LD, SD, and photoperiod-insensitive types (3, 7, 9–11, 16, 18, 19). In spite of the dramatic effect that they have on flowering, little is known about their mechanism of action. Nevertheless, some efforts have been made to gain further insight into this relatively unexplored field. To obtain maximal flowering in *Lemna gibba* G3, SA must be present in the culture medium throughout the duration of the experiment (4), thereby suggesting that its effect is probably not inductive. Takimoto and Kaihara (17) demonstrated that a short-term treatment (e.g. 24 h) with benzoic acid can initiate profuse flowering in *Lemna paucicostata* 151, provided the plants are

subsequently grown in a medium supplemented with a cytokinin, whereas a 24-h treatment with SA had virtually no effect on flower induction, even if the plants were subsequently grown in cytokinin-containing medium.

*L. paucicostata* LP6 behaves as a photoperiod-insensitive plant when grown in standard nutrient media (of different ionic strengths) such as Bonner-Devirian, Hoagland, Hutner, and Pirson-Seidel (8) that have been used routinely to culture various Lemnaceae. Flowering in strain LP6 can, however, be induced by a variety of chemicals. SA and acetylsalicylic acid are very effective (9), whereas treatment with 8-hydroxyquinoline (12), ethylenediamine-di-*o*-hydroxyphenylacetic acid (14), increased levels of iron in the simultaneous presence of cytokinins (13), and amino acids such as glutamate and aspartate (15) is less effective. Why strain LP6 is sensitive to such diverse chemicals and whether they have a common mechanism of action for induction of flowering remains to be elucidated.

SA is by far the most effective substance for induction of flowering in *L. paucicostata* LP6. In the present investigation, the effect of SA was compared to that of benzoic acid in terms of interaction with the cytokinin BAP and whether SA and benzoic acid must be present continuously to achieve the maximal flower-inducing effect. [<sup>14</sup>C]SA and [<sup>14</sup>C]benzoic acid have been utilized to determine whether the difference in relative effectiveness of SA and benzoic acid to induce flowering can be attributed to their relative uptake rates.

## MATERIALS AND METHODS

### Plant Material and General Growth Conditions

Aseptic cultures of *Lemna paucicostata* LP6 were raised in modified Bonner-Devirian medium (2), supplemented with 1% (w/v) sucrose and 10<sup>-4</sup> M EDTA. The pH was normally adjusted to 5.5 before the nutrient medium was autoclaved at 106 kPa for 15 min. SA, benzoic acid, or BAP was added to the medium before autoclaving, because autoclaving does not cause significant loss of biological activity. The plants were grown at 25  $\pm$  1°C under a mixture of cool-white fluorescent and incandescent light with a combined fluence rate from 400 to 800 nm at plant level of 22 to 27 W·m<sup>-2</sup> (4).

### Experimental Procedure

For experimental cultures, a three-frond colony was inoculated in each 250-mL Erlenmeyer flask containing 100 mL of nutrient medium. The stock and experimental cultures

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<sup>4</sup> Abbreviations: SA, salicylic acid; BAP, 6-benzylaminopurine; No. VF, number of vegetative fronds; %FL, percentage of flowering; TF No., total frond number.

were kept under a photoperiodic schedule of 16 h of light and 8 h of darkness. Usually, the experiment lasted 8 d, and flowering and growth were evaluated by %FL, No. VF, and TF No. All experiments were repeated at least once, usually several times, but data from only single, representative experiments are presented. Three replicates were kept for each treatment, and results are presented as means  $\pm$  SE.

#### Uptake of [ $^{14}$ C]SA and [ $^{14}$ C]Benzoic Acid

[7- $^{14}$ C]SA and [7- $^{14}$ C]benzoic acid were obtained from New England Nuclear, and their specific activities were 52.5 and 21.5 mCi/mmol, respectively. Four three-frond colonies were inoculated in each 125-mL Erlenmeyer flask containing 40 mL of nutrient medium (pH 5.5), and 8-d-old cultures were used to monitor the uptake of radiolabeled SA and benzoic acid. The aged medium was replaced with fresh, autoclaved medium of desired pH under aseptic conditions. Each replicate culture was supplied with 100  $\mu$ L of [ $^{14}$ C]SA or [ $^{14}$ C]benzoic acid so as to obtain 1  $\mu$ Ci per flask. Flasks were transferred to the regular growth cabinets and harvested after the desired number of hours. Methanol extracts were prepared, and the level of radioactivity was determined without further purification.

#### Extraction Procedure

The harvested plants were rinsed several times with deionized water to remove any adsorbed radioactivity on the fronds. Fronds were then ground in a mortar and pestle with a small amount of acid-washed sand and extracted overnight in cold 80% methanol. Methanol extracts were filtered and used directly for determination of radioactivity.

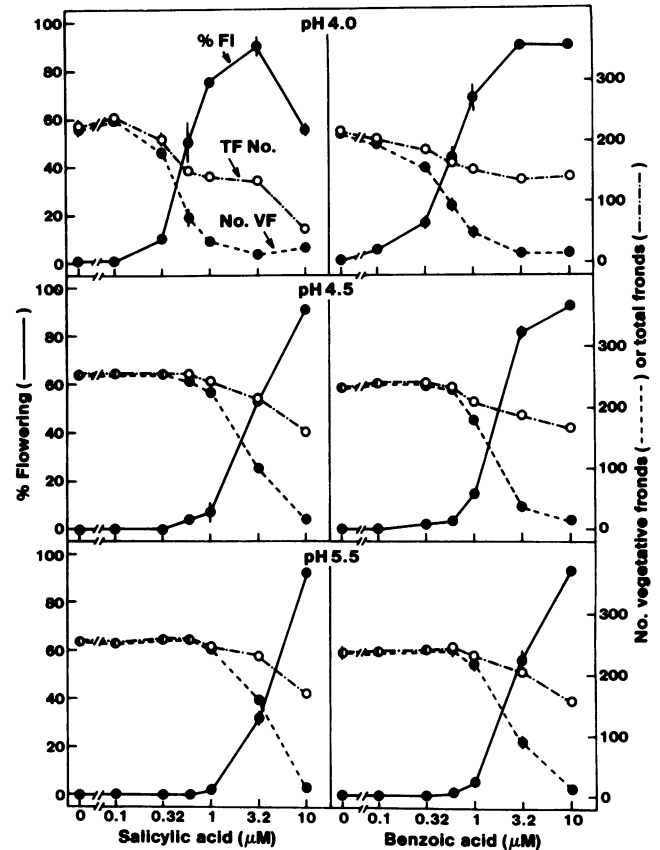
#### Scintillation Counting

Two replicate 1-mL aliquots from each sample were placed in scintillation vials, along with 9 mL of Ultrafluor (National Diagnostics). Radioactivity was determined using an LKB Rackbeta II liquid scintillation counter. A quenching curve was determined and introduced into the counter program to calculate dpm automatically from the actual cpm. The uptake experiments were repeated at least once, and each value presented is a mean of the two replicates of a representative experiment.

## RESULTS

#### Flower-Inducing Activity of SA and Benzoic Acid

The data presented in Figure 1 show that both SA and benzoic acid induce profuse flowering in *L. paucicostata* LP6, with a concomitant decrease in vegetative growth, and their effectiveness increases with decreasing pH. Benzoic acid is marginally more effective than SA; the threshold molarity of benzoic acid is slightly lower than SA, irrespective of the pH of the medium. The concentration of SA or benzoic acid required to obtain optimal flowering is relatively lower (3.2  $\mu$ M) at pH 4.0 than when the pH of the medium is kept at 4.5 or 5.5, at which 10  $\mu$ M benzoic acid or SA is optimal. Both SA- and benzoic acid-induced flowering were not influenced by the photoperiod (data not presented).



**Figure 1.** Effect of SA and benzoic acid on flowering (%FL) and growth (No. VF and TF No.) of *L. paucicostata* LP6. The pH of the medium was 4.0, 4.5, or 5.5, and all plants were grown under a photoperiodic schedule of 16 h of light and 8 h of darkness. Fronds were counted and analyzed for flowering and growth after 8 d.

#### Effect of BAP and Its Interaction with SA and Benzoic Acid

The data in Table I show that BAP (0.01–1  $\mu$ M) does not have any significant effect on either growth or flowering when *L. paucicostata* LP6 is grown in the basal Bonner-Devirian medium at pH 5.5. However, in the presence of 0.32  $\mu$ M SA, which by itself does not induce flowering, some flowering can be initiated, even with 0.1  $\mu$ M BAP, and appreciable flowering is obtained with 1  $\mu$ M BAP (Table I). A more dramatic synergism between SA and BAP is observed when the concentration of SA is increased to a suboptimal level (3.2  $\mu$ M); BAP at concentrations  $>$ 1  $\mu$ M can induce nearly 90% flowering in the simultaneous presence of 3.2  $\mu$ M SA. At higher concentrations of SA (3.2  $\mu$ M) and BAP (e.g. 1  $\mu$ M), growth is somewhat reduced, and higher levels of BAP ( $>$ 0.1  $\mu$ M) also retard the separation of daughter fronds from mother fronds, resulting in the formation of larger colonies.

In a separate experiment, the effect of different concentrations of BAP was examined in the presence of suboptimal levels of benzoic acid (0.32, 1.0, and 3.2  $\mu$ M). The data in Table II show that BAP also has a strong synergistic interaction with benzoic acid for flowering in strain LP6, and this effect was much greater than for SA (compare data in Tables I and II).

**Table I.** Interaction of SA and BAP for Their Effect on Flowering and Growth in *L. paucicostata* LP6

For experimental details see legend to Figure 1.

SA	BAP	% Fl	No. VF	TF No.
$\mu\text{M}$	$\mu\text{M}$			
0	0	0	262.0 ± 3.7	262.0 ± 3.7
0	0.01	0	289.3 ± 4.9	289.3 ± 4.9
0	0.03	0	265.6 ± 5.0	265.6 ± 5.0
0	0.10	0	266.0 ± 3.6	266.0 ± 3.6
0	0.32	0	262.6 ± 5.8	262.6 ± 5.8
0	1.0	0	259.3 ± 3.2	259.3 ± 3.2
0.32	0	0	275.0 ± 4.1	275.0 ± 4.1
0.32	0.01	0	262.0 ± 2.5	262.0 ± 2.5
0.32	0.03	0	270.6 ± 1.4	270.6 ± 1.4
0.32	0.10	2.6 ± 0.4	254.3 ± 6.1	261.3 ± 5.0
0.32	0.32	5.7 ± 0.3	233.3 ± 4.6	247.6 ± 4.4
0.32	1.0	11.9 ± 4.0	210.6 ± 4.3	239.3 ± 2.0
1.0	0	2.0 ± 0.5	275.0 ± 3.6	281.0 ± 3.7
1.0	0.01	3.4 ± 0.6	276.0 ± 4.5	286.0 ± 6.2
1.0	0.03	3.6 ± 0.5	279.6 ± 3.5	290.3 ± 3.8
1.0	0.10	22.1 ± 0.4	202.6 ± 5.7	260.3 ± 7.1
1.0	0.32	31.1 ± 1.5	165.3 ± 4.7	240.3 ± 6.3
1.0	1.0	50.7 ± 4.6	110.6 ± 9.6	225.3 ± 5.6
3.2	0	19.1 ± 2.8	219.0 ± 8.5	271.0 ± 3.0
3.2	0.01	26.9 ± 2.0	184.6 ± 5.3	254.0 ± 14.0
3.2	0.03	58.2 ± 5.2	100.2 ± 10.5	241.0 ± 4.6
3.2	0.10	88.2 ± 4.0	27.0 ± 5.5	228.6 ± 5.4
3.2	0.32	89.0 ± 4.0	24.3 ± 3.3	221.6 ± 8.7
3.2	1.0	86.7 ± 0.7	27.0 ± 1.5	202.0 ± 3.0

### Effect of 24-h Treatment with SA and Benzoic Acid

To determine whether the continuous presence of SA is essential to obtain the maximal flowering response, one three-frond colony was inoculated in each flask containing 10  $\mu\text{M}$  SA.

After 24 h, the colony was transferred to fresh medium devoid of SA, with or without 1  $\mu\text{M}$  BAP, and flowering and growth were analyzed 4 to 9 d later (Fig. 2). At pH 5.5, a short-term treatment with SA did not produce any significant effect, but flowering was enhanced by BAP when the pH of the medium was kept at 4.0. Maximal flowering (approximately 70%) was obtained 7 d after the SA treatment. Similar experiments were also performed with 10  $\mu\text{M}$  benzoic acid, and the results were similar to those for SA, except that benzoic acid elicited a much higher flowering response (Fig. 3). The effect of benzoic acid, like SA, was strongly pH dependent, and the presence of BAP was required to obtain a maximal response.

### Uptake of [ $^{14}\text{C}$ ]SA and [ $^{14}\text{C}$ ]Benzoic Acid

Plants grown for 8 d at pH 5.5, under standard conditions of 16 h of light and 8 h of darkness, were transferred to fresh medium under aseptic conditions. The pH of the fresh medium was adjusted to the desired level (4.0, 5.5, or 6.5). Uptake of [ $^{14}\text{C}$ ]SA (Fig. 4) or [ $^{14}\text{C}$ ]benzoic acid (Fig. 5) was

followed with time. The results show that there is increased uptake of both SA and benzoic acid with time, and decreasing the pH of the medium also significantly increases the uptake. Stimulation of uptake due to lowering the pH of the medium is substantially greater for benzoic acid than for SA.

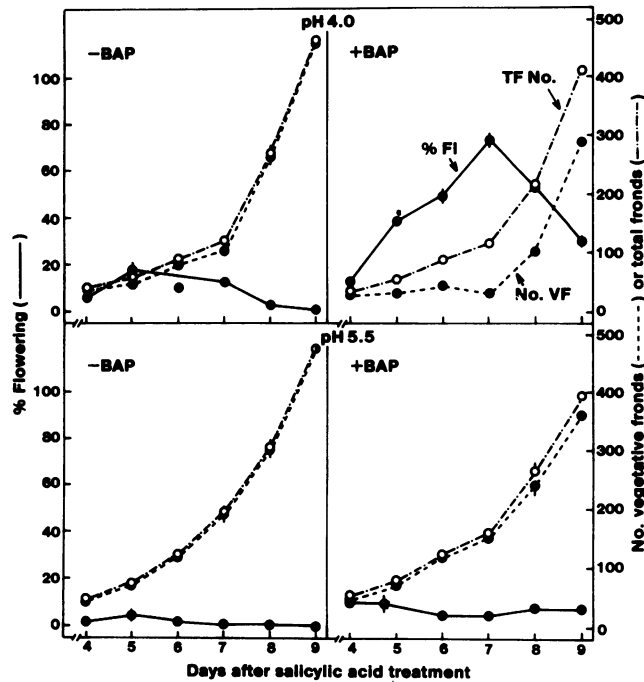
### Movement of SA and Benzoic Acid from Mother to Daughter Fronds

Experiments were designed in which an exponentially growing culture of strain LP6 was supplied either with [ $^{14}\text{C}$ ]SA or [ $^{14}\text{C}$ ]benzoic acid for 7 d, and 150 three-frond colonies were selected and transferred to basal Bonner-Devirian medium ( $\pm 1 \mu\text{M}$  BAP). These 450 fronds that were directly exposed to the radioactive medium were designated as mother fronds, and all fronds that developed subsequently were designated as daughter fronds. After 3 d, when the original three mother fronds had separated into individual colonies, the experiment was terminated and the original 450 mother fronds were separated from all daughter fronds. The mother and daughter fronds were extracted separately in 80% methanol, and radioactivity was measured in the two pools. For SA, there was a small increase in uptake of radioactivity when the pH was lowered to 4.0, but in each case an average of >99% of the radioactivity stayed with the mother fronds (Table III). The results for benzoic acid were similar but not identical. Lowering the pH to 4.0 led to a

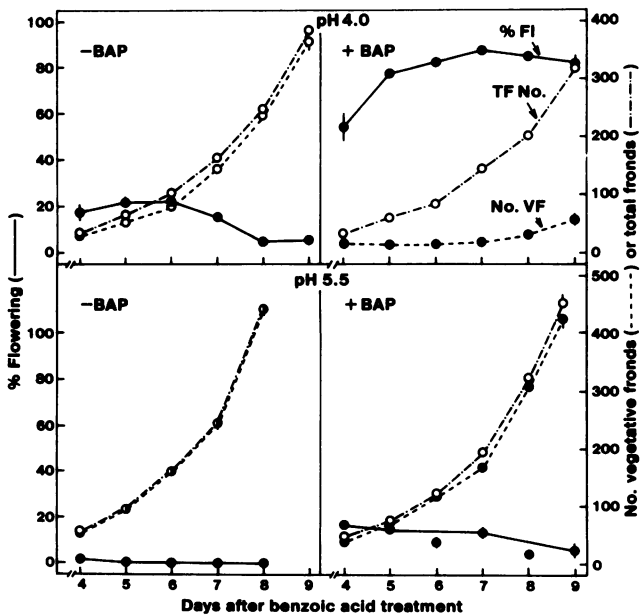
**Table II.** Interaction of Benzoic Acid and BAP for Their Effect on Flowering and Growth in *L. paucicostata* LP6

For experimental details see legend to Figure 1.

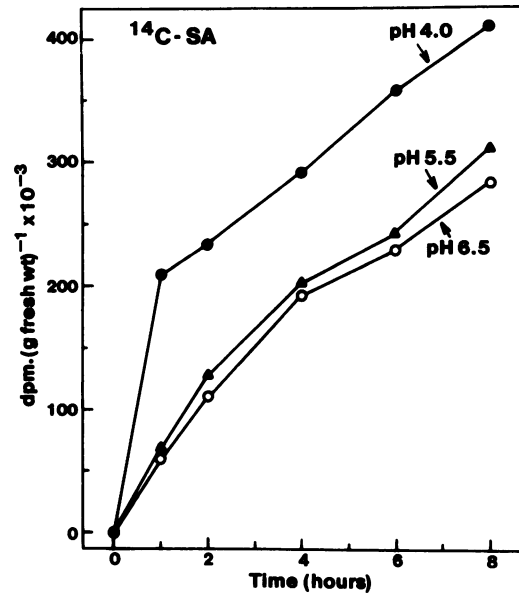
Benzoic Acid	BAP	% Fl	No. VF	TF No.
$\mu\text{M}$	$\mu\text{M}$			
0	0	0	267.3 ± 3.2	267.3 ± 3.2
0	0.01	0	252.6 ± 4.8	252.6 ± 4.8
0	0.03	0	256.6 ± 3.1	256.6 ± 3.1
0	0.10	0	245.0 ± 4.1	245.0 ± 4.1
0	0.32	0	242.0 ± 5.5	242.0 ± 5.5
0	1.0	0	234.6 ± 6.5	234.6 ± 6.6
0.32	0	0.7 ± 0.3	261.3 ± 9.2	263.3 ± 8.6
0.32	0.01	1.0 ± 0.3	260.3 ± 5.8	263.0 ± 6.2
0.32	0.03	2.1 ± 0.3	260.6 ± 1.2	266.3 ± 2.0
0.32	0.10	10.5 ± 1.8	222.0 ± 7.5	248.0 ± 3.5
0.32	0.32	14.3 ± 1.0	195.3 ± 3.8	227.3 ± 2.6
0.32	1.0	18.0 ± 1.6	191.0 ± 1.0	233.3 ± 5.7
1.0	0	8.5 ± 1.1	224.6 ± 6.8	245.6 ± 5.0
1.0	0.01	12.2 ± 1.1	200.3 ± 3.4	228.3 ± 2.4
1.0	0.03	15.2 ± 0.9	198.3 ± 3.2	234.0 ± 2.5
1.0	0.10	74.5 ± 2.3	58.0 ± 4.5	229.0 ± 4.0
1.0	0.32	79.9 ± 1.3	45.6 ± 2.9	228.0 ± 4.1
1.0	1.0	85.5 ± 4.0	30.3 ± 2.9	210.6 ± 3.9
3.2	0	36.8 ± 4.6	136.6 ± 13.6	215.6 ± 5.8
3.2	0.01	59.9 ± 4.3	87.3 ± 10.5	217.6 ± 2.7
3.2	0.03	89.3 ± 1.9	23.0 ± 4.0	216.3 ± 2.0
3.2	0.10	92.9 ± 0.6	15.3 ± 1.3	217.3 ± 2.9
3.2	0.32	93.4 ± 0.3	12.3 ± 0.3	189.6 ± 6.7
3.2	1.0	91.7 ± 0.6	15.3 ± 1.2	186.0 ± 2.0



**Figure 2.** Flowering and growth in *L. paucicostata* LP6 after a 24-h treatment with SA ( $10 \mu\text{M}$ ). After SA treatment, the plants were grown either in the basal medium (pH 4.0 or 5.5) or basal medium supplemented with  $1 \mu\text{M}$  BAP (pH 4.0 or 5.5), and flowering and growth were monitored from days 4 to 9 following SA treatment.



**Figure 3.** Flowering and growth in *L. paucicostata* LP6 after a 24-h treatment with benzoic acid ( $10 \mu\text{M}$ ). Experimental design was similar to the one described for Figure 2.



**Figure 4.** Kinetics of [ $^{14}\text{C}$ ]SA uptake by *L. paucicostata* LP6 as a function of pH of the nutrient medium. Seven-day-old cultures were supplied with radiolabeled compounds as indicated, and radioactivity was counted in methanol extracts. For more details, see text.

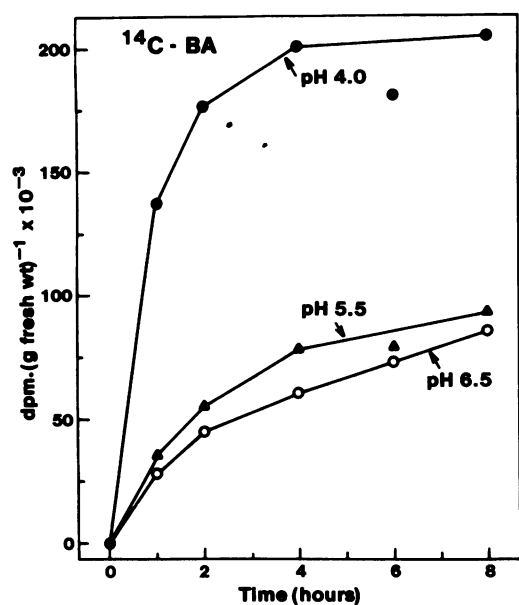
nearly 3-fold increase in uptake of radioactivity (Table III). Most of the radioactivity stayed with the mother fronds, but 4 to 5% was recovered from the daughter fronds.

## DISCUSSION

*L. paucicostata* LP6 remains vegetative when grown on the modified Bonner-Devirian medium. Substantial flowering can, however, be obtained when  $10 \mu\text{M}$  SA or benzoic acid is added to the medium, and benzoic acid is marginally but consistently more effective than SA. The threshold level of both SA and benzoic acid required for flower initiation is reduced as the pH of the medium is lowered to 4.0. Experiments in which [ $^{14}\text{C}$ ]SA and [ $^{14}\text{C}$ ]benzoic acid were used provide evidence that at pH 4.0 there is relatively more uptake of both benzoic acid and SA (Figs. 4 and 5).

These results are consistent with the fact that the protonated or undissociated form of weak acids like SA and benzoic acid will increase as the pH decreases, and because weak acids are only taken up in the protonated form, uptake will increase with decreasing pH. At a given pH the percentage of benzoic acid ( $\text{pK}_a = 4.2$ ) that exists in the protonated form will be significantly greater than for SA ( $\text{pK}_a = 2.9$ ), and this presumably explains, at least in part, why benzoic acid is more effective than SA, especially at pH 4.0 (Figs. 1–3).

In the LD plant *L. gibba* G3, the metabolism of [ $^{14}\text{C}$ ]SA was investigated, and it was shown that within 6 to 12 h a majority of the SA was converted to one or more conjugated derivatives that were presumably sequestered in the vacuole (1). The metabolism of [ $^{14}\text{C}$ ]SA and [ $^{14}\text{C}$ ]benzoic acid was not investigated in the present study, but by analogy to the earlier work with *L. gibba* G3 (1), it is likely that a sizable percentage



**Figure 5.** Kinetics of [ $^{14}\text{C}$ ]benzoic acid (BA) uptake by *L. paucicostata* LP6 as a function of pH of the nutrient medium. For experimental details see legend to Figure 4.

of both SA and benzoic acid was rapidly converted to one or more conjugated derivatives.

The presence of BAP in the basal Bonner-Devirian medium does not initiate flowering in *L. paucicostata* LP6, but it stimulates flowering when the level of iron in the nutrient medium is increased 10-fold (13). The present investigation demonstrates that BAP (when added to the basal medium) interacts synergistically with SA and benzoic acid for the stimulation of flowering in the photoperiod-insensitive strain LP6. A synergistic effect of benzoic acid and zeatin for the promotion of flowering in the SD strains 151 and 381 of *L. paucicostata* was reported earlier by Fujioka et al. (5). How-

ever, zeatin was inhibitory for benzoic acid-induced flowering in *L. gibba* G3, a LD species (6).

At pH 5.5, neither SA nor benzoic acid has an inductive effect on flowering because both substances must be present continuously to elicit a maximal flowering response. However, at pH 4.0 and in the presence of BAP, short-term treatments with both benzoic acid and SA are effective, with benzoic acid eliciting a higher flowering response than SA. Although BAP per se does not influence flowering in strain LP6, its presence was absolutely essential to accentuate the response obtained with a brief SA or benzoic acid treatment.

These results are in contrast to those obtained with *L. gibba* G3 by Cleland and Ben-Tal (4), who reported that the continuous presence of SA is required to obtain the maximal response. It should be noted that the short-term experiments with *L. gibba* G3 were conducted in  $\text{NH}_4^+$ -free half-strength Hutner's medium. Cytokinin effects were not investigated in that study, but in view of the inhibitory effect of zeatin on benzoic acid-induced flowering in *L. gibba* G3 (6), it is unlikely that addition of a cytokinin to the  $\text{NH}_4^+$ -free half-strength Hutner's medium would have resulted in the SA stimulation of flowering becoming inductive. It seems more likely that the differences between *L. gibba* G3 and *L. paucicostata* LP6 relate to the fact that *L. gibba* G3 is a LD plant and *L. paucicostata* LP6 is a photoperiod-insensitive plant.

In *L. paucicostata* strain 151, a short-term treatment with benzoic acid can induce flowering in the simultaneous presence of BAP (17), whereas a short-term treatment with SA has no effect.

In the current study, short-term treatments with both SA and benzoic acid are effective for stimulating flowering when followed by BAP treatment, but benzoic acid has a larger effect than SA. The reason for the differences in the responses of *L. paucicostata* LP6 and *L. paucicostata* 151 to short-term treatment with SA and benzoic acid is unclear.

Based on the results of experiments with strain LP6 involving short-term treatments with SA (and benzoic acid), one would presume that, after SA (and benzoic acid) is taken up

**Table III.** Level of Radioactivity ( $^{14}\text{C}$ ]SA or  $^{14}\text{C}$ ]Benzoic Acid) in Mother and Daughter Fronds

Plants were grown for 7 d in basal medium (pH 4.0 or 5.5) with  $^{14}\text{C}$ ]SA or  $^{14}\text{C}$ ]benzoic acid. At the conclusion of the 7 d, 150 three-frond colonies were selected, washed thoroughly with sterile medium to remove any adsorbed radioactivity, and transferred to basal medium (pH 4.0 or 5.5)  $\pm 1 \mu\text{M}$  BAP. Three days later, plants were harvested and separated into groups of mother and daughter fronds. Methanol extracts were prepared, and radioactivity was counted.

pH	Presence of BAP ( $1 \mu\text{M}$ )	Extract	SA			Benzoic Acid		
			Fresh wt	dpm/g fresh wt	%	Fresh wt	dpm/g fresh wt	%
			<i>g</i>			<i>g</i>		
5.5	—	Mother fronds	0.77	1,616,974	99.6	0.80	873,250	94.5
		Daughter fronds	1.95	6,153	0.4	1.95	50,292	5.5
5.5	+	Mother fronds	0.75	1,417,438	97.7	0.75	823,546	94.7
		Daughter fronds	2.13	32,853	2.3	2.00	45,690	5.3
4.0	—	Mother fronds	0.75	1,684,945	99.5	0.63	2,546,349	95.3
		Daughter fronds	2.08	8,163	0.5	1.40	124,428	4.7
4.0	+	Mother fronds	0.71	1,945,961	99.7	0.65	2,519,384	96.1
		Daughter fronds	2.01	5,825	0.3	1.61	102,565	3.9

(at low pH) by the mother fronds, either the SA (and benzoic acid) or some metabolite is translocated directly (intercellular transport) or indirectly (through the medium) to the daughter fronds and, thus, is able to induce flowering in these fronds. However, the results in Table III suggest that some other explanation is needed. For SA, there is virtually no movement of radioactivity from mother to daughter fronds, even at pH 4.0 in the presence of BAP. Clearly, something other than SA must be moving to the daughter fronds and stimulating flowering.

For benzoic acid, there is some radioactivity recovered from the daughter fronds, and although it represents only about 5% of the total radioactivity, it is significantly different from the results with SA. Apparently, a small amount of benzoic acid does move directly or indirectly to the daughter fronds, and this may explain why benzoic acid is somewhat more effective than SA for stimulating flowering when given for only 24 h. The small amount of radioactivity recovered from the daughter fronds in the case of benzoic acid, however, cannot explain the inductive effect of benzoic acid because there is no difference in the results due to pH or BAP, but a significant inductive effect by benzoic acid is seen only at pH 4.0 when the benzoic acid treatment is followed by BAP treatment.

Thus, from the present study it would appear that the effect of SA and benzoic acid on flowering in *L. paucicostata* LP6 can be of an inductive nature if the SA or benzoic acid treatment is given at pH 4.0 and is followed by BAP treatment, but the induction signal translocated from mother to daughter fronds is almost certainly not SA or benzoic acid or a direct metabolite. Because the plants were exposed to BAP following the 24-h treatment with SA or benzoic acid, the role of BAP is probably more to sustain the stimulus formed in response to SA and benzoic acid treatment, and its effect is perhaps more on the development of flowers than on the primary process of flower induction. Whether the stimulus generated by SA or benzoic acid that is translocated to the daughter fronds is similar to the photoperiodic stimulus remains to be determined.

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