$\frac{1}{8}$ -in. from the top and  $\frac{1}{4}$ -in. apart, using a  $\frac{1}{8}$ -in. and 7/64-in. drill on alternate holes. Triangular supports are glued to the back and a  $\frac{1}{4}$ -in. strip to the front, with Duco cement.

Avena seeds, germinated for 52 hr on filter paper, are placed in 18 or more of the holes in each rack. The racks are then placed in enamelware pans filled to an adequate level with tap water. The following day, obvious discards are made at the first decapitation; rigid selection within each rack may be made at the second decapitation. The seeds are somewhat smaller by this time and the 12 plants used in actual testing usually fit in the evenly spaced holes of smaller size.

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# Interaction of Auxin and Temperatures in Floral Initiation<sup>1</sup>

## A. C. Leopold and Frances S. Guernsey

# Purdue University, Lafayette, Indiana

That auxins can inhibit floral initiation has been known since the early work of Dostal and Hosek (1); conversely that auxins can sometimes induce flowering (2) or promote flowering (3) has likewise been clarified. Subsequent reports have greatly enlarged the reported instances in which auxin can inhibit or promote flowering, but in no other case than the pineapple has auxin been found actually to induce flowering. These facts suggest that plants in general may have various auxin requirements for floral initiation, and in some cases the indigenous auxin level may be supraoptimal (4) or in other cases suboptimal for flowering (3).

More recently it has been found that the effects on flowering of a given auxin treatment of pea depend to a large extent upon the subsequent temperature experience (5). The present study demonstrates that an interaction between auxin (naphthaleneacetic acid) and temperatures in floral initiation exists in all of the types of floral initiation known: that is, in photoperiodic initiation, vernalization, and indeterminate initiation of flowers. In each instance auxins can either promote or inhibit flowering, depending upon the temperature experience following the auxin treatment.

Tests with photoperiod sensitive plants included the long-day species Wintex barley, and the short-day species Biloxi soybean. Seeds were soaked in auxin solutions for 24 hr, after which they were given 18° or 3° C exposures for 2 weeks. They were then planted into the greenhouse under inductive day lengths (18

<sup>1</sup> Journal paper No. 716. Agricultural Experiment Station, Lafayette, Indiana.

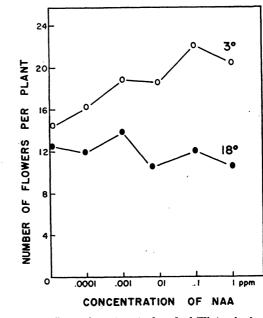


FIG. 1. Effects of treatment of seed of Wintex barley with naphthaleneacetic acid and followed by brief controlled-temperature storage (10 plants/treatment).

and 9 hr respectively). The results with the long-day barley are shown in Fig. 1, from which it can be seen that the number of flower primordia was increased over 50% by 0.1 ppm of auxin followed by the 3° C treatment; whereas, the same auxin treatment followed by  $18^{\circ}$  C treatment did not increase and may have inhibited flower initiation. The results with shortday soybean are shown in Fig. 2. It can be seen that a parallel situation holds here, in that 1 ppm auxin

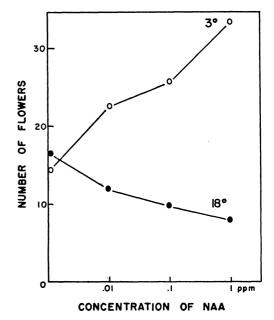


FIG. 2. Effects of treatment of seed of Biloxi soybean with naphthaleneacetic acid followed by brief controlled-temperature storage (10 plants/treatment).

treatment followed by the cool temperature more than doubled the number of flower primordia, whereas sugar and agar medium. The results of the experiflower initiation was inhibited at the warmer temperature.

Previous studies of the effects of auxin on photoperiodic induction have been applied, not as seed treatments but as leaf treatments. In order to establish whether the same responses might hold in this other type of experience, soybean plants grown to the age of 2 weeks under noninducing day lengths (18 hr) were placed in controlled temperature rooms receiving fluorescent light of 800 ft-c. They were photoinduced by five 9-hr days, one set experiencing a constant temperature of  $25 \pm 2^{\circ}$  C and the other  $10 \pm 1^{\circ}$  C during the photoinduction period. The leaf tip was removed from the youngest mature leaf of each plant and the cut surface was constantly immersed during this period in a vial containing water or auxin solutions, according to the method of Leopold and Thimann (3). At the end of the 5-day induction period the plants were transferred to the greenhouse, where they were kept on non-inducing day lengths (18 hr) until dissection at 7 weeks. The results are presented in Fig. 3, from which it can be seen that low concen-

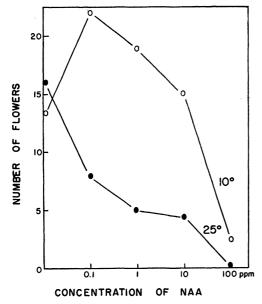


FIG. 3. Effects of treatment of leaves of soybean with naphthaleneacetic acid during photoinduction at different temperatures (10 plants/treatment).

trations of auxin (0.1-1 mg/l) promoted floral initiation at the lower temperature, whereas only inhibitions of flowering were obtained at the higher temperature. These results are strikingly parallel to the results obtained when seed-soak treatment was used.

An interaction of auxins and temperatures in vernalization has already been reported (6) with barley. Similar results have now been obtained with winter rye. Seeds were treated with auxin solutions and temperatures in the same manner as above. Simultaneous experiments were carried out with embryos excised after the auxin treatment and subsequently grown on ment are shown in Table 1, from which it can be seen

## TABLE 1

#### FLOWERING OF WINTER RYE AS AFFECTED BY TREATMENT WITH AUXIN (0.1 PPM NAPHTHALENEACETIC ACID) FOLLOWED BY BRIEF CONTROLLED-TEMPERATURE STORAGE

	No. flowers (spikelets) per plant		
	Water controls	Auxin treated	
Intact seeds, 18°	8.2	1.5	
3°	5.1	14.3	
Excised embryos, 18°	6.3	5.0	
3°	4.0	12.4	

that the auxin treatment followed by brief vernalization at low temperature nearly doubled the number of flowers, whereas the same auxin treatment at the higher temperature in the intact seeds inhibited and in the excised embryos had no effect on floral initiation. The similar responses of intact seeds and excised embryos indicates that the endosperm is not essential for the response.

The same interaction of auxins and temperatures in floral initiation of the indeterminate plant Alaska pea has already been reported (5). It was shown that auxin seed treatment followed by 10° C resulted in quantitative promotions of flowering (expressed as earliness), whereas the same auxin treatments followed by 20° C produced quantitative inhibitions of earliness.

These experiments indicate that auxins can modify floral initiation as a function of photoperiodism, vernalization, or indeterminate behavior, and that the manner of response is strikingly parallel in each case. Promotive responses to auxins were obtained in every case where short low-temperature treatments were employed, and conversely either no effect or inhibitory effects were obtained when higher temperatures were experienced. These observations are highly suggestive that the physiological mechanisms which control flower initiation by photoperiod, by vernalization, or by indeterminate means have close biochemical similarities.

Earlier workers have reported that the treatment of seeds with auxins could increase growth and flowering performance (7-9) but subsequent attempts to utilize this seed treatment as an agricultural practice failed (10). It is suggested that appropriate control of temperatures experienced by the seeds after auxin treatment may bring more consistent beneficial effects in the promotion of flowering.

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# Zinc Phosphate Identified as a Constituent of Urinary Calculi

### Jonathan Parsons

### Edsel B. Ford Institute for Medical Research, Henry Ford Hospital, Detroit, Michigan

For several years x-ray diffraction has been in use in the physics department of this institute for the analysis of urinary calculi. During this time approximately 192 stones have been studied. The method used has been described in a comprehensive paper by Prien and Frondel (1) and also in a subsequent paper by Prien (2). In general the study of calculi which has been made here agrees well with the findings of the above workers. Several different rare patterns have been obtained, however, that have not previously been recognized as possibilities. It is the purpose of this paper to report one of these findings.

In March 1951 a calculus was received measuring 39 mm along its largest axis. The stone was analyzed as principally a mixture of carbonate-apatite and magnesium ammonium phosphate hexahydrate. A totally different pattern was obtained from several yellowish-white concentric layers and from the thin outer crust (Fig. 1). This pattern was not at the time identified.

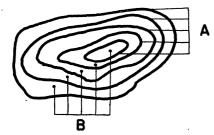


FIG. 1. Zinc phosphate in large calculus. A, zinc phosphate: B, carbonate-apatite and magnesium ammonium phosphate.

In December 1952 a 20-mm calculus was received from a different patient. The large central portion of this stone (about 80%) was yellowish white and gave a diffraction pattern identical with that of the unknown calculus pattern noted above. Surrounding the central portion was a layer of dark calcium oxalate monohydrate (Fig. 2). This dark layer was covered with a thin crust of the central material.

The fact that the relative intensities and the

FIG. 2. Zinc phosphate in small calculus. A, zinc phosphate : C, calcium oxalate.

*d*-values of the diffraction lines produced by the unknowns were the same led to the belief that they were most likely produced by a single constituent. It was recognized that different states of hydration of commonly occurring compounds might account for the appearance of this unfamiliar pattern. Spectrographic analysis<sup>1</sup> indicated zinc and phosphorus as the elements present in major proportions. X-ray diffraction interplanar spacing data<sup>2</sup> for zinc phosphate were consulted. Although the available diffraction spacings for  $Zn_3(PO_4)_2 \cdot 4H_2O$  contained fewer *d*-values than the calculus pattern, the given spacings agreed closely in *d*-values and relative intensities with the pattern lines.

For more complete verification the compound was prepared in the chemical laboratory by combining solutions of the soluble salts—zinc chloride and sodium orthophosphate. After the precipitate was washed well and recrystallized from orthophosphoric acid, it gave a diffraction pattern which was identical with that of the unknown calculus powder. Table 1 shows clearly the close agreement of the calculus pattern and that of the prepared  $Zn_3(PO_4)_2 \cdot 4H_2O$ .

No explanation for the urolithiases of the zinc phosphate calculi described in this paper has been offered.

### TABLE 1

#### COMPARISON OF X-RAY DIFFRACTION POWDER PATTERN INTERPLANAR SPACINGS\*

Only lines with relative intensities of 4 and above have been included in this table. In all, 63 lines with d values down to 0.905 have been checked and confirm the identification of the pattern.

Calculus*		Prepared powder†		Calculus		Prepared powder	
d‡	$I/I_1$ §	$\overline{d}$	$I/I_1$	d	$I/I_1$	d	$I/I_1$
9.21	9	9.17	9	2.61	4	2.61	6
5.32	4	5.32	5			2.54	5
5.08	5	5.11	5	2.53	4	2.51	5
4.86	6	4.86	6	2.27	<b>5</b>	2.27	6
4.59	7	4.58	8	2.10	4	2.10	5
4.42	6	4.42	7	2.01	4	2.01	6
3.99	5	3.99	6	1.94	6	1.96	7
3.88	4	3.88	4	1.83	<b>5</b>	1.83	6
3.47	<b>5</b>	3.47	6	1.57	<b>5</b>	1.57	6
3.39	8	3.39	8	1.53	4	1.53	5
2.86	<b>10</b>	2.86	10	1.51	4	1.51	5
2.65	4	2.65	5				

\* Unknown calculus pattern.

† Chemically prepared  $Zn_3(PO_4)_2 \cdot 4H_2O$ . ‡'Values in angstrom units.

§ Relative intensity (visual estimation).

<sup>1</sup> This analysis was made through the courtesy of the Research Laboratories División, General Motors Corporation.

 $^{\rm 2}$  Interplanar spacing cards prepared by American Society for Testing Materials, 1950 edition.