

since shock was used during the tests, the experiment provided no information about the nature of the gradient following training to a single stimulus; nor did it provide information about the changes in the shape of the gradient during the extinction of suppression.

The sharpening of the generalization gradient, during extinction, has also been reported in several studies involving the galvanic skin response (3), though in none were the effects as large as those herein described. It is perhaps of importance, however, that the methodology involved in the conditioned suppression paradigm and that involved in conditioning the galvanic skin response is similar. In both procedures, a noxious event is paired with a neutral stimulus.

Of the large number of other studies of stimulus generalization, only a few have reported data on changes in the gradient during extinction (4), and these indicate a variety of different results. At present the reasons for these differences are not clear (5).

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Distribution of Strontium-90 in a 1959 Wheat Sample

Abstract. At least 22 percent of the strontium-90 found in a sample of wheat harvested in 1959 was due to direct deposition. Twenty-seven percent of the total strontium-90 content of this wheat sample was contained in the outermost bran layer.

Knowledge of the mechanism by which plants are contaminated by fallout is essential for the accurate prediction of future levels of Sr^{90} in foods. A particularly important fact that must be known is whether plants become contaminated principally from Sr^{90} that is absorbed from the soil or from Sr^{90} that results from the direct deposition of fallout on the plant surfaces. Studies presented here on the contamination of wheat may be of some help in resolving this problem.

Analyses, conducted by the Health

Table 1. Distribution of strontium-90, stable strontium, and calcium in a sample of hard red Kansas wheat.

Per- cent of total	Strontium-90		Stable strontium		Calcium	
	$\mu\mu\text{c}$ in fraction	$\mu\mu\text{c}/\text{kg}$ of fraction	mg in fraction	mg/kg of fraction	g in fraction	g/kg of fraction
100.0	47.2	47.2	<i>Original wheat kernel</i>			
			3.15	3.15	0.49	0.49
95.8	36.2	37.8	<i>Stripped wheat</i>			
			2.95	3.08	0.42	0.44
4.2	12.7	302.0	<i>Epidermis</i>			
			0.18	4.29	0.07	1.65
	+1.7 (4%)		<i>Material balance</i>			
			-0.02 (1%)		0.00 (0%)	

and Safety Laboratory (1), of wheat and its milling products from 1958 and 1959 crops in the United States showed that the highest Sr^{90} concentration occurred in the bran fraction of wheat kernels. Similar results were reported by British investigators of the Agricultural Research Council Laboratory (2) for wheat grown in the United Kingdom and other countries in 1958.

The root uptake of Sr^{90} by wheat plants has been studied by C. C. Lee (3) in greenhouse experiments. He found that Sr^{90} absorbed from the soil is also concentrated in the bran.

Since the bran comprises the outer layers of the wheat kernel, it would be expected that much of the Sr^{90} found in this fraction is due to direct deposition. Lee's experiments indicate, however, that some of the bran contamination of field-grown wheat may result from root uptake.

Any directly deposited Sr^{90} that enters the wheat kernel must first pass through the outermost bran layer or epidermis. It is reasonable to expect, therefore, that a high proportion of the Sr^{90} in this layer is due to direct deposition. Experiments performed at the Health and Safety Laboratory on a sample of Ohio wheat harvested in 1958 showed that 25 percent of its Sr^{90} content was in the epidermis while this fraction constituted only 4 percent of the total weight of the wheat. To determine the origin of the Sr^{90} in the epidermal fraction of wheat kernels, further experiments measuring the ratio of Sr^{90} to stable strontium (specific activity) in the epidermis and in the remaining portion of wheat were conducted on a sample of Kansas wheat.

The separation of the epidermis was done by Theodore Earle (4) by a method, devised by him, which utilizes the friction produced in the controlled agitation of a mixture of grain and water to strip the epidermis from the wheat kernels.

Table 1 lists the results of analyses for Sr^{90} , stable strontium, and calcium

performed on (i) a sample of hard red Kansas wheat, harvested in 1959, (ii) the epidermis stripped from the wheat, and (iii) the residual stripped wheat. The values tabulated are estimated to be accurate to within 10 percent.

The material balances obtained indicate that the Sr^{90} , stable strontium, and calcium were not removed by the water used in the stripping operation.

Although strontium and calcium are chemically similar, they need not be physiologically identical. From data in Table 1, the ratio of stable strontium to calcium is found to be $0.18/0.07 = 2.6$ mg/g in the epidermis, while this ratio is $2.95/0.42 = 7.0$ mg/g in the stripped wheat. The difference in these ratios is indicative of dissimilar metabolic pathways for the soil-derived stable strontium and calcium. It is reasonable to conclude from these data that relatively little of the Sr^{90} found in the epidermis came from the soil.

Of the Sr^{90} found in the epidermis, that amount derived from root uptake may be estimated quantitatively by calculating the specific activity of the stripped wheat. If one assumes that the plant cannot distinguish stable strontium from Sr^{90} in the process of root absorption, the specific activity (Sr^{90} to stable strontium) of all parts of the wheat kernel should be the same. The specific activity of the stripped wheat is $36.2/2.95 = 12.3$ $\mu\mu\text{c}$ of Sr^{90} per milligram of stable strontium. This would be the specific activity of the epidermis, if there had been no direct deposition. The observed specific activity of the epidermis was $12.7/0.18 = 70.6$ $\mu\mu\text{c}$ of Sr^{90} per milligram of stable strontium; hence it appears that there must have been some direct deposition of Sr^{90} . The stable-strontium content of the epidermis was 0.18 mg; therefore, $12.3 \times 0.18 = 2.2$ $\mu\mu\text{c}$ of Sr^{90} must have resulted from root uptake. This is a maximum estimate since it has been assumed that all of the activity in the stripped wheat came from the

soil and none came by migration from the directly deposited Sr^{90} on the epidermis.

The epidermis contained 12.7 $\mu\mu\text{c}$ of Sr^{90} . If 2.2 $\mu\mu\text{c}$ of this total is a maximum estimate of the amount due to root uptake, then at least 10.5 $\mu\mu\text{c}$ must have been due to direct deposition. The total Sr^{90} content of the entire wheat kernel was 47.2 $\mu\mu\text{c}$. Hence at least 22 percent of this total can be ascribed to direct deposition.

If the findings of this experiment can be generalized, a substantial drop in the Sr^{90} content of the 1960 wheat crop can be expected since the fallout rate and presumably the direct deposition of Sr^{90} on plants was much less in 1960 than in 1959 (5).

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5. Many of the radiochemical analyses reported here were done by Joseph Catania, Analytical Division, Health and Safety Laboratory. It is a pleasure to acknowledge his assistance.

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Preparation of a Floral

Initiating Extract from *Xanthium*

Abstract. An extract from lyophilized tissue of flowering *Xanthium* (cocklebur) plants initiates floral development when applied to *Xanthium* test plants maintained on long-day photoperiodic conditions. Details of the preparation of the extract are described.

Hamner and Bonner (1) have submitted the first evidence of a hormone formed in *Xanthium* at the site of photoperiodic perception, the leaf, and later utilized by the bud in the transition from the vegetative to the flowering condition. They also record attempts to find a floral initiating preparation in extracts from flowering plant material. At the same time they tested numerous chemicals for activity. Although they did occasionally find evidence of floral-initiating activity in their plant extracts, they were unable to demonstrate any consistent pattern or to repeat their results with any confidence.

Table 1. Flowering response of *Xanthium* after application of extract.

Flowering response	Numerical av. of flowering response (6)
<i>Untreated control plants</i>	
All ten plants vegetative	0.0
<i>Extract-treated plants</i>	
Five plants vegetative (one plant, stage one; two plants, stage two; two plants, stage three)	1.1

Since that time there have been occasional published reports of preparations exhibiting floral-initiating properties. Such reports have not met the test of consistent repetition, with the exception of the now well-known effect of gibberellic acid in stimulating floral development in many long-day photoperiodically sensitive plants. Lang (2) suggests that gibberellic acid is probably not the primary floral-initiating stimulus, but that it acts indirectly in stimulating flowering. The action of gibberellic acid in promoting more rapid flowering in *Xanthium* (3) also appears to be an indirect effect.

The experiment described in the present report is the last of four successful demonstrations of floral initiation in *Xanthium* plants resulting from applications of as yet unknown substance(s) extracted from the branch parts of flowering plants. Our preparation has been independently checked by B. H. Carpenter and K. C. Hamner (4), and the results are in accord with our own.

Flowering branch tips were harvested from indigenous *Xanthium strumarium* L. var. *canadense* (Mill.) T. and G. (5) of the Long Beach area. The plants were picked when the staminate terminal inflorescence was from $\frac{1}{2}$ to 1 cm in diameter. Each branch carried three to five mature leaves. The fresh material was frozen in liquid nitrogen and broken into fine fragments while in the frozen state. Care was taken that the material remained frozen until it had been thoroughly dried in a laboratory vacuum lyophilizer. After lyophilization, the material was placed in sealed containers and stored in a deep freeze at -20°C .

The lyophilized material was extracted with absolute methanol under a partial vacuum sufficient to maintain the temperature of boiling methanol between -5° and -10°C . A modified Soxhlet apparatus was used with a condenser containing a dry ice-acetone slurry as a coolant. The methanol solvent was removed from the extract by evaporation at room temperature or below in a Rinco apparatus attached to

a water aspirator system. The product was a dark green, tarry residue which was mixed to homogeneity with anhydrous (USP) lanolin.

Two grams of the residue mixed with 17 g of lanolin was applied to the underside of the leaf surface of ten test plants. Ten control plants were treated in like fashion with 17 g of pure lanolin. During the 14 days subsequent to the application of the lanolin preparations, all test plants and control plants were maintained on a precise 8-hour dark and 16-hour (500 ft-ca intensity) light regime. On the 14th day after application, the terminal bud of each plant was dissected to ascertain the flowering response. The flowering stages were numerically evaluated (6), and the results are presented in Table 1. Three earlier experiments had yielded essentially similar results.

It is our belief that these results constitute the first reproducible demonstrations of floral initiation in a short-day plant as the direct result of an extract prepared from the tissues of flowering plants. It is suggested that this is a crude extract of the flowering stimulus. Currently, efforts are being directed toward further concentration and characterization of the active entity in this extract (7).

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4. A preparation of 2 g of extract mixed in 17 g of lanolin was given to B. H. Carpenter and K. C. Hamner, Botany Department, University of California at Los Angeles. The mixture was applied to ten test plants of *Xanthium*; ten comparable plants served as controls. All plants were maintained on long-day conditions for 17 days, after which they were dissected. The treated plants were flowering at the 50-percent level. All controls were vegetative.
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6. Results were evaluated in accordance with a numerical scale based on the diameter and morphological stage of development of the terminal staminate inflorescence. Vegetative plants were rated as zero on the scale. The first morphological change in the stem apex that could be clearly recognized as flowering was assigned a value of 1.0. A flowering apex measuring 0.25 mm in diameter was evaluated as 2.0. An additional increment of 1.0 was allowed for each 0.25 mm increase in the diameter of the developing inflorescence.
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