Table III. Distribution of P^{32} in Seed Kernels from P^{32}-Labeled DEF-Treated Plants as Compared to Unlabeled Phosphorus Distribution in Control Plants

	Treated Plants		Control Plants	
Phosphorus Fraction	P^{32}, c.p.m. per gram[a]	Total P^{32} in fractions, %	P, mg. per gram	Total P in fractions, %
Inorganic	1125	61.2	0.34	3.2
Phytin	540	29.4	8.80	82.6
Carbohydrate ester	0	0.0	0.07	0.7
Phosphatide	44	2.4	0.62	5.8
Nucleic acid	130	7.1	0.82	7.7
Total P[b]	1839	100.1	10.65	100.0
Separate determination	1835		11.15	

[a] Corrected for self-absorption. Standard error of radioactive count between duplicates of samples not over 5%.
[b] Sum of individual fractions.

Table IV. Distribution of S^{35} in Seed Kernels from S^{35}-Labeled DEF-Treated Plants as Compared to Unlabeled Sulfur Distribution in Control Plants

	Treated Plants		Control Plants	
Sulfur Fraction	S^{35}, c.p.m. per gram[a]	Total S^{35} in fractions, %	S, mg. per gram	Total S in fractions, %
Soluble organic (nonprotein)	265	8.3	0.146	9.6
Soluble sulfate	5	0.2	0.063	4.2
Insoluble sulfate	266	8.4	0.165	10.9
Insoluble organic (protein)	2649	83.2	1.140	75.3
Total sulfur[b]	3185	100.1	1.514	100.0
Separate determination	3120		...	

[a] Corrected for self-absorption. Standard error of radioactive count between duplicate samples not over 5%.
[b] Sum of individual fractions.

as was phosphorus. Apparently the increase of radioactivity in the cottonseed kernel after treatment with labeled-DEF is due mainly to breakdown products of the DEF and not from the unaltered defoliant itself. The concentration of the latter in the developing kernel remains at a constant level (0.2 to 0.3 p.p.m.).

Entrance of DEF into the kernels from surface contamination is somewhat higher in this case than in actual practice since the open bolls were harvested 5 hours after spraying instead of 7 to 10 days after defoliation, as is customary. Contamination could also be minimized by using a suitable method of kernel processing. More investigations should be carried out since it is not known conclusively at present whether DEF or its breakdown products (*8*) are toxic with respect to cholinesterase in vitro.

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FUNGICIDE EFFECTS ON FLAVOR

The Effect of Some Fungicides on the Flavor of Canned Strawberries

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OFF-FLAVOR in canned strawberries has been attributed to captan and thylate. Tapio (*9*) mentioned captan induces in strawberries a "bitter flavor" which persists in canned fruit and jam. Marsh *et al.* (*6*) were unable to detect captan in canned fruit. Crang and Clarke (*2*) found 11 to 19 p.p.m. captan and an undescribed off-flavor in canned fruit in one year.

Strawberries sprayed with thiram were found by Marsh *et al.* (*6*) to have a marked off-flavor when canned, while Tapio (*9*) found no off-flavor. Crang and Clarke (*2*) report definite off-flavor in canned fruit in two years out of five, although certain individuals were able to detect differences in all trials.

The objective of the present study was to determine whether off-flavors were detectable in strawberries that were canned subsequent to field treatment with Phaltan, captan, or Thylate. The ability of individuals to detect off-flavor due to added fungicide was also determined.

Materials and Methods

In 1960 and 1961, Sparkle strawberries were sprayed with Phaltan (50W, 50% *N*-trichloromethylthiophthalimide), captan (50W, 50% *N*-trichloromethylthio-4-cyclohexene-1, 2-dicarboximide), or Thylate (65% bisdimethylthiocarbamoyl disulfide). The rate of application

In two consecutive years, strawberries sprayed with captan or thiram for mold control did not develop off-flavors when canned in plain or enamelled cans. In one year, an off-flavor was found in fruit which had been sprayed with Phaltan and canned in unlined cans. Captan added to sweetened strawberry juice was detected at 5 p.p.m. and Phaltan, at 25 p.p.m. Thylate was not tasted at concentrations up to 25 p.p.m. Berries carrying residues of 6.5 p.p.m. captan or 13.1 p.p.m. thiram had less than 1 p.p.m. fungicide after canning by the cold-pack method.

was 4 pounds per 200 gallons per acre. Sprays were applied when blossom buds were visible, when one third of the blossoms were open, at petal fall, and 10 days after petal fall. Sprayed plots and unsprayed checks were randomized in four blocks.

Samples of berries from each plot were collected at intervals of 0, 3, and 9 days after final spraying and preserved for residue analysis by freezing at −10° F. without further treatment or by canning in sucrose sirup by the cold-pack method. In 1962, the samples of canned and frozen berries were analyzed for both Thylate and captan. Methods of the A.O.A.C. (*1*) were followed, except that Thylate was stripped from the fruit with benzene rather than chloroform, as decomposition of thiram in chloroform extracts was very rapid.

In both years, the remainder of the fruit picked on the third day following the final spray application was bulked, hulled, and canned (unwashed) in 45% sucrose sirup by the cold-pack method. Two complete series were prepared—one in plain and the other in fruit enamel cans (IIIZ Continental Can Co.). All canned fruit was stored at 77° to 80° F.

In 1960, the canned fruit was tasted after 3 months in storage; in 1961, fruit was sampled after 2 days and after 2, 5, 9, and 12 months in storage. At each sampling period, cans were torn down and examined for visible signs of corrosion.

The canned fruit was compared for flavor differences by methods similar to those suggested by Wiley *et al.* (*11*), McArdle *et al.* (*5*), and Murphy *et al.* (*7*). Three cans of each treatment were taken at random from storage. The entire contents was comminuted and divided into 1-ounce samples. The samples of all fungicidal treatments were coded and submitted to each taster together with a labeled reference standard prepared from unsprayed fruit. Samples from one type of can were assessed in morning sessions, and those from the other in the afternoon. Tests were repeated on three consecutive days in 1960 and four consecutive days in 1961. These tests were considered to be replications. In 1960, a coded check was not included, but in 1961 a coded sample from the same lot as the standard was submitted as an unknown.

Each of 18 volunteer tasters was asked to compare the flavor of each coded sample to the standard and indicate his assessment—by checking the appropriate square of the score sheet (*7*)—as better than standard, equal to standard, inferior to standard but not off-flavor, slightly off, or definitely off. Scores were subsequently assigned to these assessments on a 5-point scale ranging from +2 to −2 (*4*).

Since human beings are inclined to think there is a difference if the possibility of a difference is suggested, some measure of this factor must be made. Serving two identical samples—the labeled standard and the coded unknown from the same can—affords an opportunity to evaluate this factor. The scores of each volunteer taster were examined to determine the frequency with which he correctly scored the coded check throughout the 1961 experiment (40 tastings). A Chi-squared test was applied to determine departure from chance distribution. Three judges correctly scored the coded check at a probability level of 0.001. These three judges were selected as the taste panel, and only their scores were considered in determining the presence or absence of off-flavor in the treated samples.

When the results showed that the selected panel was unable to establish differences in flavor between treated and untreated canned fruit, their ability to detect the fungicides was tested by the following method. Juice was extracted from untreated strawberries by cooking and pressing. Sucrose was added at 45% by weight. Fungicides were added to the juice to give concentrations of 0, 5, 10, 15, 20, and 25 p.p.m. The dilution series of each fungicide was submitted separately, along with an identified "standard" containing no fungicide, to each judge. Judges were requested to compare each sample to the standard. Code numbers were jumbled. No more than one series was tested at any one session. The experiment was replicated four times. Judges' reactions were recorded, scored, and analyzed.

Results

In 1960, the flavor scores of strawberries canned after field treatment with various fungicides showed differences which were significant at the 1% level of probability (Table I). These differences were associated with the fungicide and not with can enamel. The enamel × fungicide interaction was significant.

Table I. Analysis of Variance of Taste Panel Scores Assigned to Canned Strawberries (1960)

Source of Variation	Degrees of Freedom	Mean Square	Variance Ratio, F
Treatments	5	2.02	4.12[a]
Fungicides	2	2.90	5.82[a]
Enamels	1	0.90	1.84
F × E	2	1.69	3.45[b]
Judges	2	0.35	0.71
Replication	2	1.15	2.35
Remainder	44	0.49	
Total	53		

[a] Significant at $P = 0.01$.
[b] Significant at $P = 0.05$.

Table II. Mean Taste Panel Scores Assigned to Strawberries Receiving Various Field Treatments Prior to Canning in Plain or Enamelled Cans (1960)

Treatment	Mean Score	$\lvert 1 - x \rvert$	Significance of Difference
Phaltan, plain	−0.33	1.33	0.001
Phaltan, enamel	0.55	0.45	0.20
Captan, plain	1.00	0	0.50
Captan, enamel	0.68	0.32	0.20
Thylate, plain	0.67	0.33	0.20
Thylate, enamel	0.89	0.11	0.11

When treatment mean scores were compared (Table II), only Phaltan plain had the negative score considered by Murphy *et al.* (*7*) to be indicative of off-flavor. This flavor was identified by panel members as "tinny" or "metallic," and not characteristic of the fungicide. Etching or corrosion of the container was not evident.

In 1961, differences between treatments were not significant at the 5% level, but highly significant differences appeared between storage periods. There were no significant interactions between fungicides, can linings, and storage periods. Mean scores for treatments did not differ significantly from the mean scores for the corresponding untreated checks (Table III). No visible corrosion was noted on the con-

Table III. Difference in Mean Scores of Canned Strawberries Sprayed with Fungicide Prior to Canning and Similar Unsprayed Berries at Different Storage Periods (1961)

	Plain Cans					Enamelled Cans				
		Months					Months			
Difference	2 Days	2	5	9	12	2 Days	2	5	9	12
Phaltan *vs.* check	0.08	0.34	0	0.16	0.08	0	0.09	0	0.09	0.09
Captan *vs.* check	0	0.25	0.21	0.09	0.09	0.08	0.17	0.50	0.09	0
Thylate *vs.* check	0	0.34	0.58	0.16	0	0.08	0.09	0	0.08	0
L.S.D. ($P = 0.05$)	0.11	0.52	0.69	0.50	0.25	0.17	0.36	0.52	0.44	0.36

Table V. Mean Panel Scores Assigned to Strawberry Purees to which Fungicide Was Added after Cooking

	Concentration, P.P.M.						
Fungicide	0	5	10	15	20	25	D[a]
Phaltan	0.92	0.75	0.58	0.58	0.33	0.25	0.66
Captan	0.92	−0.25	−0.42	−0.67	−0.84	−0.92	0.66
Thylate	0.92	0.75	1.00	0.92	0.75	1.00	0.48

[a] Tukey's confidence interval at 5% probability (*8*).

tainer walls or ends at any period up to 1 year of storage at 77° to 80° F.

Fungicide residues on the fruit harvested and frozen immediately after spraying were relatively high but decreased as the interval between application and harvest increased (Table IV). At the 3-day interval, there were 6.5 p.p.m. captan and 13.1 p.p.m. thiram. The canned fruit, on the other hand, contained less than 1 p.p.m. of fungicide, even when canned immediately after spraying. Recovery tests for fungicides added to canned strawberries after processing gave average values of 103.2% for captan and 82.4% for Thylate.

When the fungicides were added to sweetened strawberry puree subsequent to cooking, the panel was unable to detect any flavor difference as a result of the addition of Thylate in concentrations up to 25 p.p.m. (Table V).

Mean panel scores for purees containing Phaltan were inversely related to fungicide concentration, but a statistically significant difference between treated and untreated puree did not occur until the 25 p.p.m. level had been reached.

When captan was added to the cooked, sweetened strawberry puree, the mean panel scores for treatment decreased as the concentration increased. The scores showed differences between 0 and 5 p.p.m. captan, but no differences between 10 and 25 p.p.m.

The off-flavor in the captan and Phaltan series was characterized as "earthy" or "musty" when detected, with some individuals finding a suggestion of "metallic."

Discussion

Off-flavors, associated with the addition of captan, Thylate, or Phaltan to cooked, sweetened strawberry puree, were not readily discernible, and not all individuals were reliable in detecting the presence of the fungicide. This is in agreement with the findings of Crang and Clarke (*2*). Taste thresholds were relatively high, possibly higher than the residues normally present on harvested, washed fruit. The practical importance of such off-flavors is questionable.

Sprayed berries had residues of 6.5 p.p.m. captan or 13.1 p.p.m. Thylate when harvested 3 days after the final spray. This is in agreement with Fahey *et al.* (*3*) and Marsh *et al.* (*6*). Negligible residues were found in canned fruit and no off-flavors were detected which could be associated with these two fungicides.

Crang and Clarke (*2*) state that in 1954 canned fruit contained 11 to 15 p.p.m. captan. This appears to be a misstatement of the findings of Marsh *et al.* (*6*), who report that this quantity was present on fresh fruit, but "captan was not found" in the canned strawberries. They suggest the off-flavor may be caused by a decomposition product.

While no off-flavors caused by Thylate or captan were found in the canned fruit in the present study, possibly larger residues on the raw fruit could lead to their development in storage. Since there apparently is some change in the fungicides during canning, these off-flavors could be associated with a degradation product or a reaction involving the can lining.

Table IV. Fungicidal Residues on Strawberries (1961)

Fungicide	Days after Spraying	Residue on Fruit, P.P.M. Frozen	Canned
Captan	0	14.8	<0.2
	3	6.5	<0.2
	9	3.8	<0.2
Thylate	0	58.3	<1.0
	3	13.1	<1.0
	9	21.2	<1.0

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