of the 2,4-D acid remaining on one piece of glass and 475 μg of the 2,4-D ethyl ester on the other. During the following 3-day period, there was no measurable loss in weight of the 2,4-D acid sample, but the sample of 2,4-D ethyl ester lost 86% of its original weight. The temperature varied from 79° to 90° F but was about 85° during most of this period.

With the exception of Amo 1618, the rates of evaporation of those chemicals that were previously applied to tinfoil were also measured after they had been applied in a similar manner to glass. The period of evaporation was 24 hr, and the temperature varied from 85° to 88° F. In this experiment, approximately equal amounts of each compound were applied. The carbamate Amo 1618 did not adhere to the surface of the glass, and therefore tinfoil was used in place of glass. Results of these later experiments were similar to the earlier ones with tinfoil. IPC and 3-Cl-IPC both evaporated at a very rapid rate, whereas an appreciable amount of α-naphthalenacetic acid did not evaporate. As would be expected, the 2,4-D ethyl ester

TABLE 1

EVAPORATION OF SOME GROWTH-REGULATING COMPOUNDS DURING THE FIRST 24-HR PERIOD AFTER BEING FINELY DISPERSED ON GLASS SLIDES HAVING A SURFACE AREA OF 25 CM²

Compound	Temperature range (°F)	Amount used (μg)	Percentage evaporation in 24 hr
3-Cl-IPC	85–88	364	91.9
IPC	85–88	382	79.6
2,4-D ethyl ester	79–90	475	29.0
α-Naphthalenacetic acid	85–88	492	5.5
2,4-D acid	79–90	343	0
Amo 1618*	79–90	4651	0

* Amo 1618 applied to tinfoil instead of glass.

evaporated, and the 2,4-D acid failed to do so. On the other hand, the ethyl ester did not evaporate nearly as fast as did either the IPC or the 3-Cl-IPC (Table 1).

These experiments show that certain carbamates evaporate at a relatively rapid rate at moderate or comparatively high temperatures. This would seem to indicate that when they are applied in small amounts their effectiveness as plant growth regulators might be reduced through rapid evaporation. Rhodes *et al.* (*3*) reported that the sprout-inhibiting effect of isopropyl-N-phenyl carbamate decreased when treated potato tubers were exposed to air. Marth and Schultz (*4*) reported that relatively small amounts of 3-Cl-IPC inhibited the sprouting of potatoes stored at room temperatures, but only when the treated tubers were temporarily stored at low temperatures (40°–50° F) prior to storage at the higher temperatures (about 70°–75° F). In their experiments an average of approximately 4.5 mg of the 3-Cl-IPC was applied per tuber. It would be expected, on the basis of the present results, that practically all of this would evaporate during the first 24-hr period following its application unless it penetrated into the tuber. Temporary storage at low temperatures apparently allowed time for the tubers to absorb an effective amount of the compound before it evaporated.

Some growth-regulating substances are effective when applied to plants in minute amounts, even at relatively high temperatures, since they apparently do not evaporate at a sufficient rate to reduce their effectiveness. For example, apple drop, which sometimes occurs before harvest, may be greatly reduced by the application of sprays that contain only 10 ppm α-naphthalenacetic acid. In supplementary experiments the acid was applied at this rate to mature apples, and the fruits retained an average of only about 4 μg of the chemical.

Stevens and Carlson (*5*) reported that temperatures below 75° F markedly delayed the disappearance of 3-chloro-isopropyl-N-phenyl carbamate following its application to soil. It would appear from the present results that, when growth-regulating compounds such as IPC and 3-Cl-IPC are used as herbicides at high temperatures, they may evaporate in sufficient amounts to reduce their effectiveness.

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The Antithyroid Activity of some Compounds that Inhibit Peroxidase

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The percutaneous absorption of resorcinol has been reported to induce myxedema with goiter in human subjects (*1*), and the parenteral administration of this substance to the rat acutely depressed the accumulation of radioactive iodine by the thyroid gland (*2*). In a recent study of many polyhydric phenols Arnott and Doniach (*3*) showed that compounds containing hydroxyl groups *meta* to one another (e.g., resorcinol, phloroglucinol, hydroxyhydroquinone) were most effective in decreasing iodine uptake by the rat thyroid, and these authors suggested, on the basis of an earlier observation of an inhibitory action of resorcinol on milk peroxidase (*4*), that the antithyroid effect of substances of this kind might be ascribed to inhibition of a thyroid peroxidase. Their report prompts us to record experiments along similar lines performed in

this laboratory. The effects, described below, of certain phenols and amines on peroxidase activity and thyroid function lend support to the view that peroxidase inhibitors have antithyroid effects and, by implication, favor the concept that a thyroid peroxidase may be concerned with the organic binding of iodine.

The assay of antithyroid effect was performed by the method of McGinty (*5*). The test substance, in aqueous or dilute ethanol solution, was injected subcutaneously in rats weighing 100–150 g; 1 hr later, 6–8 μc I^{131} was administered intraperitoneally, and 4 hr after this the animals were killed, the thyroids removed and dissolved in hot NaOH. The radioactivity was determined by γ-ray counting of the liquid sample (*6*). The results for each compound were expressed as a percentage of the uptake in control animals run simultaneously. The effect of some of the compounds on peroxidase was studied by a modification of Randall's manometric procedure (*7*). The enzyme was prepared from raw milk by Elliot's method (*8*) and dried from the frozen state after removal of salts by dialysis; aqueous solutions were made before use. A mixture of enzyme, substrate (*p*-phenylenediamine, catechol, or benzidine in final concentration 0.003 *M*), hydrogen peroxide (.003 *M*) in phosphate buffer (pH 6.8) was incubated in the main compartment of Warburg vessels with and without test compounds, which were used in concentration 3–20% of the substrate; after 5–15 min catalase was tipped in from the side arm, and residual peroxide was thus determined. Qualitative tests of the antiperoxidase effect were also made by adding equal volumes of a peroxidase-peroxide-buffer solution to each of a series of tubes containing progressive tenfold dilutions of the test substance; after a few minutes a starch-iodide solution was added and the rate of iodine color development observed.

The effects of the test substances on thyroid uptake of I^{131} are indicated in Table 1. The considerable inhibitory effect of phloroglucinol confirms the observation of Arnott and Doniach (*3*); the much less marked effect of resorcinol noted in the present studies may be related to the longer time interval between injection of the compound and sacrifice of the animal. Phenol, catechol, pyrogallol, α-naphthol, and vanillin did not depress thyroid uptake of radioactive iodine. Among the amines tested, *m*-phenylenediamine, in contrast to the *o*- and *p*-isomers, exhibited marked antithyroid effect. The toxicity of *p*-phenylenediamine (10–15 mg being a fatal dose) precluded its use in dosage comparable to the other isomers. *p*-Aminophenol did not inhibit uptake, whereas *m*-aminophenol did so strikingly, and, similarly, 4- but not 5-aminosalicylic acid decreased thyroid I^{131} accumulation. The amino-analog of phloroglucinol was somewhat less effective than the latter compound. Aniline and the three isomeric toluidines induced great depression of iodine uptake.

The ratio of I^{131} concentration in thyroid and serum of animals pretreated with propylthiouracil and then receiving phloroglucinol or aniline did not differ from that of controls receiving propylthiouracil alone, indicating that these substances inhibit organic binding of iodine by the thyroid and do not affect the iodide-concentrating mechanism.

TABLE 1*

	No. of rats	Dose (mg/rat)	Thyroid I^{131} accumulation % of controls	Inhibition of peroxidase
Resorcinol	3	2	76	++
	4	10	50	
	2	25	74	
Phloroglucinol	3	2	25	++
	4	5	26	
	2	25	14	
Pyrogallol	3	7	90	–
	4	8	94	
	2	25	95	
m-Phenylenediamine dihydrochloride	2	2	32	++
	3	5	53	
	3	25	6	
	3	30	13	
p-Phenylenediamine dihydrochloride	2	2	84	–
	3	6	76	
o-Phenylenediamine dihydrochloride	3	25	64	–
m-Aminophenol	5	25	10	+
p-Aminophenol	5	25	93	–
4-Aminosalicylic acid	5	25	34	+
5-Aminosalicylic acid	3	25	122	–
1,3,5-Triaminobenzene hydrochloride	2	10	48	
	3	15	39	
Aniline	3	20	7	++
m-Toluidine	5	25	14	++
o-Toluidine	5	25	12	+
p-Toluidine	3	25	10	++

* In the last column, (–) indicates no inhibition, (+) and (++) indicate, respectively, moderate and marked inhibition.

The peroxidase experiments showed that resorcinol, phloroglucinol, *m*-aminophenol, *m*-phenylenediamine, 4-aminosalicylic acid, aniline, and the toluidines significantly inhibited the enzymic oxidation of the substrate. Although *o*-toluidine was a substrate for peroxidase as judged by color development, the compound decreased the rate of peroxide disappearance when *p*-phenylenediamine or catechol was present as a substrate. Those substances mentioned above which did not inhibit thyroidal iodine accumulation (phenol, catechol, pyrogallol, 5-aminosalicylic acid, vanillin) appeared to be good substrates of peroxidase. The studies of Balls and Hale (*9*) disclosed a number of substances, some generally considered to be substrates of vegetable peroxidase, which in the presence of peroxide decreased the activity of the enzyme preparations. It is of interest that among peroxidase inhibitors they listed aniline, phloroglucinol, *m*- and *p*-toluidine, and resorcinol, compounds which have marked antithyroid activity. The *meta* configuration, in the case of polyphenols and amines, would appear to be particularly active in both respects.

It is thus a curious fact that most classes of antithyroid substances are either competitive substrates or inhibitors of peroxidase, the thiocarbonamides being in the former category (*7*) and sulfonamides (*10*), anilines, and polyphenols in the latter.

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Ascorbic Acid and Physiological Breakdown in the Fruits of the Pineapple (*Ananas comosus* L. Merr.)

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Many tropical fruits may be injured when they are stored too long at temperatures in the range of 0°–10° C. The injury is often called "chilling," and it is usually characterized by a darkening of the flesh or of the peel and by failures of the mature green fruits to ripen properly when they are subsequently stored at room temperature. Specific effects on flavor and chemical constituents may also appear, depending upon the particular kind of fruit involved.

The exact manner in which chilling modifies the normal metabolism of tropical fruits is not entirely understood, although several investigators have contributed valuable information to the subject. Jones (*1*), in his studies of the papaya, found that ripening changes (especially hydrolysis of sucrose) were retarded by low temperatures, and the effect on respiration was so marked as to suggest that the basic metabolism of the fruit was upset. Harris and Poland (*2*) reported a loss of ascorbic acid in chilled bananas, but the fruits had been severely injured by exposure to low temperatures, so that these particular changes may have been largely the result of autolysis of the cells. It was previously reported by the senior author (*3*) that two lots of imported pineapples showing physiological breakdown were characterized by a lower content of ascorbic acid than was true of normal fruits. These fruits showed advanced stages of physiological breakdown, and, as in the experiments of Harris and Poland, the destruction of ascorbic acid may have occurred during autolysis rather than in the earlier stages of exposure to low temperatures. The results that are now being reported were obtained by analyzing pineapples which had been stored at low temperature but which had not shown visible evidence of physiological breakdown when the fruits were sampled.

TABLE 1

EFFECT OF COLD STORAGE ON CERTAIN CONSTITUENTS OF THE JUICE OF THE ABACHI PINEAPPLE

Treatment	Bromelin activity ($1/t$)	Total soluble solids (%)	Total acid (g/100 ml)	Ascorbic acid (g/100 ml)
Stored at room temperature	0.377	12.9	0.98	42.4
Stored at 6° C for 1 wk, then at room temperature for 2 days	0.363	11.5	0.79	25.9

Abachi pineapples were grown in Florida, harvested in the "mature green" or "market ripe" stage of maturity and shipped to Pittsburgh, Pa., by railway express. Upon arrival at destination the fruits were divided into two lots, each comparable to the other in regard to stage of maturity of the individual fruits. One lot was held at room temperature (25°–30° C) for 2 days and then analyzed. The other lot was stored at 6° C for 1 week and then held at room temperature for 2 days before analyzing. Each fruit was sampled individually in order to facilitate statistical interpretation of the results. The expressed and filtered juice was analyzed for total soluble solids, total acids, ascorbic acid, and bromelin activity. Each lot consisted of 10 pineapples. The averaged results appear in Table 1.

The two lots did not differ significantly in regard to total soluble solids, total acids, and bromelin activity of the juice, but a highly significant difference in ascorbic acid was observed. The refrigerated lots of pineapples contained 25.9 mg ascorbic acid/100 ml juice, compared to 42.4 mg in the control fruits. This amounts to a reduction of 38.9%.

The pineapples showed no ill effects of the cold storage other than this loss of ascorbic acid. There was no discoloration of the flesh and no deleterious effect on flavor. In other words, the period of storage was interrupted before any visible symptoms of chilling had appeared, and it is concluded that destruction of ascorbic acid constitutes the first phase in the development of low-temperature injury.

This does not explain all that occurs when tropical fruits are injured by exposure to low temperatures. It *does* suggest that such a treatment interferes with a specific step in the respiratory processes of the plant cell. It is known, for example, that in one stage of respiration certain phenolic compounds are oxidized to quinones, the latter being black or brown in color. During the normal course of the process quinones are converted back to phenols by ascorbic acid. This reversible action continues as long as an adequate supply of ascorbic acid is present. Since physiological breakdown of pineapples is characterized by a darkening of the flesh, it seems logical to conclude that the discoloration in the affected fruits indicates that the