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Received for review October 25, 1957. Accepted May 7, 1958. Approved for publication by the director of the Wisconsin Agricultural Experiment Station.

FUNGICIDE RESIDUES

Determination of Tetrachloro-1,4-benzoquinone (Spergon) Residues on Food Crops

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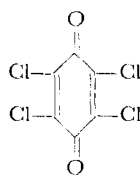
Spergon is dissolved from plant surfaces with benzene. The Spergon in the recovered solvent oxidizes diphenyl-p-phenylenediamine to a blue Wurster salt. Extraction of the benzene with an aqueous acetic acid–hydrochloric acid solution removes the Wurster salt to the aqueous acid phase. The absorbance is measured spectrophotometrically at 700 mμ. This method has been applied to broccoli, cauliflower, and lettuce. Recovery data were obtained at the 0.1- and 0.5-p.p.m. level.

The fungicidal properties of tetrachloro-1,4-benzoquinone (Spergon) were discovered at the General Laboratories of United States Rubber Co. in 1940 (*8*).

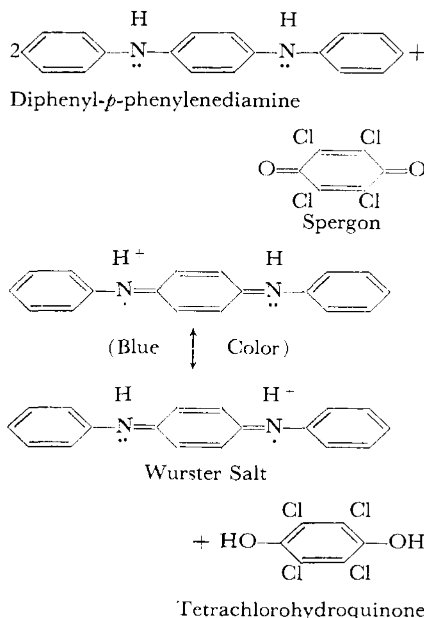


Spergon

Spergon is used chiefly to protect seeds and bulbs (*1, 4, 6, 7*). It also gives outstanding control of downy mildew of broccoli, cauliflower, and lettuce (*1, 5, 6*). The latter use involves spray or dust application to food crops, which necessitates residue analysis to determine a safe level of use—in the neighborhood of 0.1 p.p.m.

Several good color tests for microdetermination of Spergon are known. For example, Spergon forms a strong yellow color with anhydrous diethylamine, which is the basis for the determination of Spergon on treated seed (*3*). In aqueous sodium hydroxide, Spergon readily converts to the sodium salt of chloranilic acid. Upon acidification, the free chloranilic acid produced is an intense purple color. These color tests are sufficiently sensitive for residue analysis; however, attempts to recover 1-p.p.m. doses of Spergon from broccoli and cauliflower by these tests failed.

Compounds of the same general type as Spergon combine irreversibly with plant constituents. The failure of the above color tests, which utilize the reactive chlorine atoms of Spergon, to produce color indicated that the reactive chlorine atoms of Spergon had been rendered inactive by combination with plant constituents. Next, attempts were made to utilize the oxidative properties of the quinone groups of Spergon to produce a sensitive color, and they were successful. Diphenyl-*p*-phenylenediamine is readily subject to oxidation by mild oxidizing agents such as Spergon (benzoquinones) to produce an intense blue color owing to the formation of a Wurster salt (*9*).



The Wurster salt is a resonating free radical with several possible resonant forms. The two major contributing forms are shown above.

When untreated broccoli or cauliflower was treated with fractions of 1 p.p.m. of Spergon, good recoveries were obtained by use of the diphenyl-*p*-phenylenediamine color test (Table I). The oxidative property of the quinone groups of Spergon was not hindered by the reaction of other parts of the molecule with plant constituents. The bound Spergon remains benzene-soluble and dissolvable from the plant surfaces.

Experimental

Apparatus. Spectrophotometer, Beckman Model DU equipped with 1-cm. matched Corex or silica cells.

Reagents. Diphenyl-*p*-phenylenediamine. Purify by vacuum distillation of crude material. Collect the white crystalline plates and store in a covered jar.

Diphenyl-*p*-phenylenediamine reagent. Dissolve 0.5 gram of purified diphenyl-*p*-phenylenediamine in 50 ml. of benzene. Store in a brown glass bottle. Prepare fresh daily.

Extracting solution. In a suitable acid bottle, place 850 ml. of glacial acetic acid, 100 ml. of distilled water, and 50 ml. of concentrated hydrochloric acid. (Deaerate daily before using by bubbling dry nitrogen gas through the solution for 0.5 hour.)

Processing of Sample. Place a 1000-gram sample of the crop and a volume

(in milliliters) of benzene (technical grade) equal to one half the sample weight in a suitable glass gallon jar, protecting the contents from the lid by a piece of cellophane or polyethylene sheet. Roll the jar on an automatic rolling device for 15 minutes to dissolve the Spergon from the surfaces of the sample. Decant the benzene and scrub it twice with 100-ml. portions of distilled water to remove water-soluble oxidizing agents which are sometimes present and interfere. Dry the benzene over anhydrous sodium sulfate and filter through a rapid filter paper.

Procedure. Transfer 400 ml. of the filtered benzene wash to a 500-ml. separatory funnel. Add 5 ml. of the diphenyl-*p*-phenylenediamine reagent. Shake vigorously for 1 minute and let stand for 4 minutes. (The oxidation of the diphenyl-*p*-phenylenediamine by the Spergon as shown above takes place at this point, appearing as a weak, yellowish-orange color.) Next, add 50 ml. of the extracting solution, shake thoroughly for 1 minute, and let stand 1 to 2 minutes for layer separation. The Wurster salt partitions into the aqueous acid phase as a blue color in approximately 10 ml. of solution—the solvent-phase readjustment accounts for the apparent loss of the remainder of the 50 ml. of extracting solution added. Draw off the blue-colored aqueous acid layer into a 1-cm. cell and measure the absorbance at 700 mμ using extracting solution as the reference (Figure 1), 10 minutes after addition of the diphenyl-*p*-phenylenediamine reagent to the 400 ml. of benzene. If the blue solution is foggy, filter through E-D No. 613 filter paper, or equivalent, before measuring the absorbance. The intensity of the blue color increases with time—hence the stringent time element is in the method.

The increase of intensity is fairly constant with any series of analyses, averaging 0.003 p.p.m. per 5-minute interval. To minimize the inherent error of the increase of color from spectrophotometric measurements, make three analyses simultaneously (the limit of the four-cell spectrophotometer carriage). By use of extracting solution as the reference, run a reagent blank analysis to determine background color and its rate of increase, together with either duplicate treated analyses or an untreated and an untreated plus known. From a standard curve, determine the microgram equivalent of absorbances found.

Calculations. Convert the absorbance difference between a sample analysis and its reagent blank analysis to micrograms from the standard curve. The micrograms found divided by the sample weight equivalent to the amount of benzene wash used for analysis gives apparent parts per million.

$$\text{P.p.m., treated sample (corrected)} = \frac{\text{apparent p.p.m., treated sample} - \text{apparent p.p.m., untreated sample}}{\text{\% recovery of known}} \times 100$$

Standard Curve. Run a standard curve of absorbance *vs.* concentration of Spergon at 0.1, 0.2, 0.3, and 0.4 p.p.m. —i.e., 80, 160, 240, and 320 γ per 400 ml. of benzene—just prior to the analysis of samples or whenever a new batch of extracting solution is used. A standard analysis of 80 γ per 400 ml. of benzene minus reagent blank analysis gives an absorbance of approximately 0.3 (Figure 2). In the event initial analyses on treated samples are in excess of the absorbance values available from the standard curve, dilute the blue solution with extracting solution to reduce the absorbance to the usable range of the standard curve. In future analyses on this sample, dilute the benzene wash with benzene to the extent that a 400-ml. aliquot will produce color intensity within the useful range of the standard curve.

Although individual standard curves follow Beer's law, the slopes of curves obtained from the use of different batches of extracting solution vary slightly. The limits of variance of 12 typical standard curves are shown in Figure 2. From the average standard curve, the extremes of variance are ±7%. The extracting solution—85 parts of acetic acid, 5 parts of hydrochloric acid, and 10 parts of water—provides optimum conditions for the removal of the Wurster salt to the aqueous acid phase. Varying the proportions of acetic acid, hydrochloric acid, or water reduces considerably the amount of blue color extractable from the benzene. For a large series of analyses, one large bottle of extracting solution from which to draw helps to keep at a minimum the number of standard curves to be run.

Discussion

Procedure. All solutions used in analysis should be about 70° to 80° F. because the oxidation of the diphenyl-*p*-phenylenediamine by Spergon is retarded substantially at lower temperatures.

Two means of adding known amounts of Spergon to untreated samples were used. Initially, the benzene and known amount of Spergon were added to the untreated sample in a glass jar and tumbled on an automatic rolling device. Later, the untreated sample was washed with benzene alone, the known amount of Spergon was added to an aliquot of the recovered solvent. Recoveries of knowns were similar from each manner of addition.

Processing of Sample. To ensure adequate washing of the broccoli and cauliflower heads, the closely packed blossoms were cut loose from the main stem as were the lateral shoots before the samples were tumbled in benzene. In the case of broccoli and cauliflower, the solvent not only washes the plant surfaces, but also dissolves the surface coating of natural wax and penetrates the general exterior 0.5 to 1 cm.

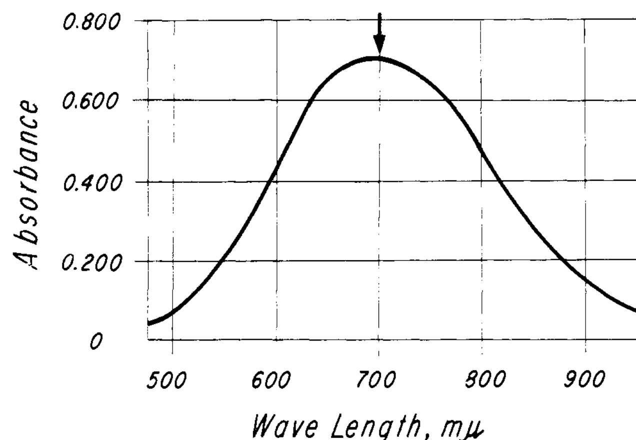


Figure 1. Absorption spectrum of the blue color produced by the oxidation of diphenyl-p-phenylenediamine by Spergon

Arrow indicates choice wave length

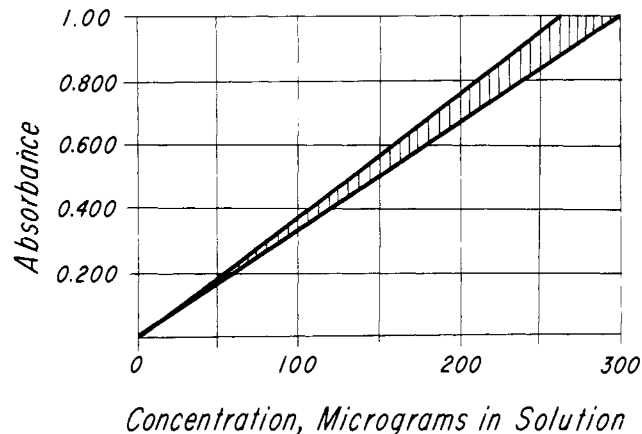


Figure 2. Effect of various batches of extracting solution on color intensity of standard curves

Table I. Recovery Data

	Added, P.P.M.	Recovered, P.P.M.[a]	Recovery, %
Broccoli	0.52	0.45	87
	0.50	0.41	82
	0.50	0.49	98
	0.50	0.47	94
	0.50	0.37	74
	0.50	0.40	85
	0.10	0.11	110
Cauliflower	0.50	0.37	74
	0.50	0.36	72
	0.55	0.41	74
	0.51	0.44	86
	0.55	0.41	75
Lettuce	0.10	0.09	90
	0.96	1.10	115
Av.			86

[a] All values given are corrected for background interferences due to untreated samples.

Crop Interference. Interference coloration produced by untreated samples of lettuce range from 0 to 0.1 p.p.m., broccoli from 0.05 to 0.25 p.p.m., and cauliflower 0.2 to 0.4 p.p.m. The higher interference values obtained from broccoli and cauliflower were from samples received overripe, in flower, and in some cases somewhat dehydrated. Samples in marketable condition gave the lower interference values.

Bruce (*2*) has shown that interfering materials in the benzene strip solutions of broccoli, cabbage, and cauliflower can be removed by adding 10 grams of a mixture of anhydrous sodium sulfate, Attapulgas clay, and Filter-Cel (2 to 2 to 1 by weight) per 100 ml. of strip solution. The purifying agents are removed by filtering the mixture through a rapid paper. Consistently good recoveries of Spergon were obtained when amounts as low as 0.06 p.p.m. were added to untreated samples.

Acknowledgment

The author is indebted to Agricultural Chemical Group of Naugatuck Chemical for their assistance and cooperation in making this work possible and to Dorothy R. Hill for many of the experimental data obtained and presented in this paper.

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Received for review April 8, 1957. Accepted April 30, 1958. Division of Analytical Chemistry, 131st Meeting, ACS, Miami, Fla. April 1957.

ALGICIDE MEASUREMENT

The Microdetermination of 2,3-Dichloro-1,4-naphthoquinone (Phygon) in Water

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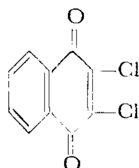
A spectrophotometric method for the determination of micro amounts of Phygon in water is described. The insoluble matter in the water is removed by filtration, the filtrate is adjusted to an acid pH by the addition of phosphoric acid, and the Phygon is distilled from the filtrate. Chloroform extraction of the distillate concentrates the Phygon, and the evaporation of the chloroform to a small volume results in a further concentration. The Phygon in the chloroform is detected by its ultraviolet absorption spectrum. Correction for background of the absorption data reduces the error introduced by other ultraviolet absorbing materials. This method is applicable in the range of 8 to 250 parts per billion, with an average recovery of 86%.

PHYGON (*5*) has been used widely as a foliage spray to control diseases caused by fungi on a variety of food crops.



Phygon (Dichlone, 2,3-dichloro-1,4-naphthoquinone)

The quinone structure of Phygon and its successful use as a fungicide led Fitzgerald, Gerloff, and Skoog (*7*) and Fitzgerald and Skoog (*2*) at the University of Wisconsin to include it in their screening tests for toxicants of bloom producing blue-green algae.

Since this work of Fitzgerald and Skoog, the application of Phygon as an algicide and submersed aquatic weed regulant has received wide and successful use. Some of its uses have been in recreational lakes, farm ponds, irrigation reservoirs, swimming pools, industrial water, and recirculating systems.

Depending upon the species of algae and type of aquatic weeds that are to be controlled, doses from 0.03 to 0.75 p.p.m. have been effective (*4*). Excellent control of algae growth in the recirculating cooling towers at the Naugatuck Chemical plants at Naugatuck, Conn., and Baton Rouge, La., has been obtained over a 2-year period with a concentration of 0.25 p.p.m. This level is maintained by daily additions of Phygon and routine analysis of the cooling tower water by the method described herein.

Phygon has a strong ultraviolet absorption spectrum in chloroform (Figure 1). Micro quantities [parts per billion