Scott, W. P.; McKibben, G. H. *J. Econ. Entomol.* **1978**, *71*, 343.
Su, H. C. F. *J. Econ. Entomol.* **1977**, *70*, 18.
Su H. C. F. *J. Ga. Entomol. Soc.* **1978**, *13*, 269.
Su, H. C. F., Stored-Product Insects Research and Development Laboratory, SEA, USDA, Savannah, GA, unpublished data, 1979.
Synerholm, M. E.; Hartzell, A.; Arthur, J. M. *Contrib. Boyce Thompson Inst.* **1945**, *13*, 433.

Received for review July 16, 1980. Accepted September 29, 1980. Names of companies of proprietary products are given solely for the purpose of providing specific information; their mention does not imply recommendation or endorsement by the U.S. Department of Agriculture over others not mentioned.

A Rapid Colorimetric Method for Analysis of Carbaryl Spray Deposits on Fruit Tree Foliage

Mikio Chiba

A rapid colorimetric method was developed to determine deposits of carbaryl insecticide on fruit tree foliage. Analyses take less than 3 min/sample when 50 or more samples are processed at a time. A 5-cm^2 disk punched from a leaf is used for the determination. Carbaryl is extracted and hydrolyzed by methanolic NaOH (0.03% w/v) and then coupled with *p*-nitrobenzenediazonium tetrafluoroborate which produces a spectrum of colors ranging from red to blue. Within a concentration range of 0.5–10 μg/cm^2 of leaf surface or 0.25–5 μg/mL of alkaline solution in a test tube, the absorbance of color obeyed Beer's law when measured at 580 nm. Little, if any, interference was observed from other commonly used pesticides, such as dicofol, tetradifon, azinphosmethyl, phosmet, captan, and folpet. If a spectrophotometer is not available or when a rapid field test is required, a semiquantitative determination is also possible.

Methodology for pesticide residue analysis has tended to emphasize greater sensitivity and specificity by using sophisticated techniques and instruments. Automated analysis is one technique well suited for analyzing large numbers of samples, especially on a routine basis (Gunther and Ott, 1966). In many laboratories, however, automated systems are not available because they are costly and much experience is needed to set up complete analytical systems.

To date, methods for determining spray deposits are generally those used for the measuring of pesticide residues in foodstuffs. Without exception, these analyses are based on "adequate sampling in a large quantity", sufficient to give reliable average values. This approach is essential in ordinary residue analyses. However, for assessment of the performance of sprayers, relative to uniformity of distribution and amount of chemical applied to the target, analyses of individual leaves are essential. The importance of individual analyses was explained and a special extraction apparatus was developed by Pielou et al. (1962). Chiba (1973) and Chiba et al. (1973) demonstrated that without individual analyses, differences in amount and distribution of deposit between leaves within a limited area and in different locations of a tree cannot be identified. Individual values, rather than a single average value, can provide answers to questions that were previously impossible to answer. Ordinary residue methods are not usually suitable for handling large numbers of samples in a limited period of time. The method described in this paper was developed to permit rapid determination of carbaryl (1-naphthyl methylcarbamate) spray deposits on leaves of fruit trees and grapevines. The original request came from field entomologists who needed an unsophisticated method that could be used by a nonchemist. Such a method could be used to judge whether another spray application is necessary after a heavy rainfall or to assess insect damage relative to the distribution of deposits on target trees or vines. Although this concept is different from the usual approach to pesticide residue analysis, the color reaction employed is basically the same as that in the TLC method described by Chiba and Morley (1964).

With this method residues on individual leaf disks may be measured by a relatively inexperienced person in less than 3 min/sample when 50 or more samples are processed at a time. In contrast, the official Association of Official Agricultural Chemists (1965) method in the hands of an experienced operator requires more than 1 h/sample when six (or eight) samples are processed together.

This new method requires only a leaf punch and a colorimeter or a spectrophotometer in a laboratory for accurate measurement. A simple semiquantitative determination can be made anywhere, however, by matching the color with a series of color standards. This is possible because the reaction product yields a spectrum ranging from red to blue, depending on concentration. The effective range of concentration is 0.25–5 μg/mL in alkaline solution.

MATERIALS AND METHODS

Apparatus Employed. *Spectrophotometers*: Spectronic 20 (Bausch & Lomb, Inc., Rochester, NY 14625) and DK-2A (Beckman Instruments, Inc., Fullerton, CA 92634). *Pour-out dispensers*: 20-mL capacity for 0.03% NaOH in methanol solution; 1-mL capacity for the chromogenic reagent (Arthur H. Thomas Co., Philadelphia, PA 19105). *Test tubes*: disposable culture tube, 18 × 150 mm (Kimble Products, Toledo, OH 43601). *Test tube caps*: 2030 cap, 17 mm (Falcon Plastics, Oxnard, CA 93030). *Leaf punch*: 2.523-cm diameter (5.00 cm^2 disk). Manufactured locally; an improved version of the unit reported previously (Gordon and Little, 1954).

Reagents. *Carbaryl*: 99% plus, analytical standard (Union Carbide Corp., Salinas CA 93901); Sevin 50% WP (wettable powder) (Niagara Chemicals, Burlington, Ontario L7S 1W6). *p-Nitrobenzenediazonium tetrafluoroborate*

Agriculture Canada, Research Station, Vineland Station, Ontario LOR 2EO, Canada.

0021-8561/81/1429-0118$01.00/0

(*NBDFB*): practical grade, P7078 (Eastman Organic Chemicals, Rochester, NY 14650). *NaOH and 1-naphthol*: analar grade (BDH, Toronto, Ontario M8Z 1K5). *Methanol and acetone*: ACS grade (BDH, Toronto, Ontario M8Z 1K5).

Analytical Methods. Twenty milliliters each of 0.03% NaOH in methanol (w/v) solution in selected test tubes is prepared. One 5-cm^2 leaf disk from leaves of sprayed fruit trees or grapevines is added to each tube and immersed in NaOH solution by using glass rods (one/tube). After two min the leaf disks are removed by using the glass rods and then there is a 30 min wait.

The NBDFB solution is prepared by dissolving 50 mg of NBDFB in 50 mL of acetone in a 100-mL pour-out dispenser flask and adding 50 mL of methanol; within 10 min 1 mL is added to each test tube. (Gelatin capsules, No. 000, Parke Davis Co., Ltd., Brockville, Ontario, are convenient containers for preweighed 50-mg quantities of NBDFB. For preparation of NBDFB solution, the capsule is opened, the capsule and contents are dropped into the flask, and solvents are added. The capsule does not dissolve.)

Each tube is covered with a plastic cap and mixed well by tumbling end over end or by using an appropriate vibrator. After 10 min the absorbance of each tube is measured at 580 nm. The reference blank consists of the color developed with a nonsprayed leaf disk. The total quantity of carbaryl (micrograms) in the test tube is determined with a calibration curve prepared as follows.

Disks from nonsprayed leaves are punched and immersed in 20 mL each of 0.03% NaOH in methanol solution containing 5, 10, 20, 30, 40, 50, 60, 80, and 100 μg of carbaryl in individual test tubes. At 580 nm the Beer–Lambert law is obeyed in the above range of carbaryl concentrations.

FACTORS STUDIED

Alkaline Concentration. The effect of alkali concentration (0.02, 0.03, 0.05, 0.1, 0.2, 0.3, and 0.5%) in methanol on factors such as extraction and hydrolysis time, linearity, and color stability were investigated. In addition, the effect of water at 5, 10, and 50% in the methanol alkaline solution was assessed.

NBDFB Solution. Different volumes (0.5–3.0 mL) and concentrations of NBDFB solutions (0.025–0.1%), prepared with acetone, methanol, and a mixture of the two (1:1) were assessed from effect on color appearance, stability, and linearity of the color produced.

Time Required for Extraction and Hydrolysis. The time required to extract quantitatively and complete hydrolysis of carbaryl was investigated at intervals ranging from 10 s to 5 min for extraction and from 5 to 60 min for hydrolysis. Completeness of extraction and hydrolysis was judged by the standard colors produced with 1-naphthol in the concentration range stoichiometrically equivalent to that of the carbaryl standards prepared.

Color Spectrum. Absorption spectra of the solutions with the color developed were examined with the scanning Beckman spectrophotometer by using pure CH_3OH and extracts of untreated leaf as reference blanks. All experiments to assess the above factors were carried out with the Beckman instrument, but the Spectronic 20 spectrophotometer was used for the routine analyses.

Color Stability. Absorbance of the color was measured immediately after color development and at intervals up to a maximum of 16 h to see the stability of the colors developed.

Test Tubes as Cuvettes. The color reaction was completed with a large volume of carbaryl solution (2.2 μg/mL) which after development was divided into 40 test tubes. The absorbance of each test tube was first recorded to determine extent of variation. Each tube was then rotated in the cell holder of the Spectronic 20, and the tube was marked to indicate the position that yielded the most uniform absorbance in the series (e.g., 0.580). The absorbance of each tube was then read a third time by positioning the tubes according to the marks.

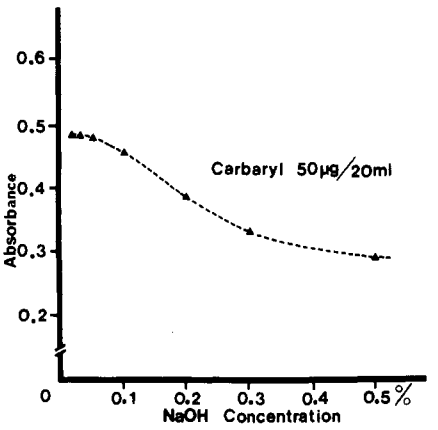

Figure 1. Absorbance at 580 nm of 50 μg of carbaryl and 1 mL of NBDFB solution in 20 mL of methanol relative to NaOH concentrations.

Interference from Other Chemicals. The presence of 1-naphthol, a probable degradation compound of carbaryl on sprayed foliage, was checked by the thin-layer chromatographic method (Chiba and Morley, 1964). The method was also tested with 2 μg/mL, of such common pesticides as dicofol, DDT, tetradifon, azinphosmethyl, phosmet, captan, and folpet individually with and without addition of carbaryl solution (2 μg/mL) to assess possible interference.

Leaf Factor. The effects of species (apple, pear, peach, and grapes) and leaf age on background absorbance and possible interference were examined.

Comparison with the AOAC Method. Five hundred leaves were taken from the bottom outside area of a sprayed peach tree (carbaryl 50% WP, 2 g/L and 13.5 L/tree), and one disk was punched from the center of each leaf. The disks were mixed well and subdivided into groups of 50. Seven groups (350 disks) were analyzed individually by this method; the remaining three groups were analyzed by the Association of Official Agricultural Chemists (1965) official method.

RESULTS AND DISCUSSION

Experimental Parameters. Intensity of the color developed was influenced by the concentration of alkali. As the alkali concentrations increased, absorbance decreased (Figure 1). Absorbance recorded with 0.2 and 0.5% NaOH in methanol solutions was about 80 and 60%, respectively, of that with 0.03% NaOH solution. Moreover, sodium hydroxide must be dissolved in CH_3OH only. If water was used at even 5% v/v in CH_3OH to dissolve NaOH quickly, both pH of the solution and the time required to complete the hydrolysis increased substantially.

The time required to complete the hydrolysis of carbaryl was slightly longer with 0.03% NaOH than with 0.2% NaOH (Figure 2). The 0.03% solution was chosen in this method, however, because it gave slightly stronger absorbance and better linearity than did 0.2% NaOH in the concentration range of 5–20 μg/20 mL. The 0.2% NaOH solution is a good choice though when time is really limited.

Excellent linearity with the curve passing through the origin was achieved at all the concentrations. Linearity and reproducibility were only possible, however, when

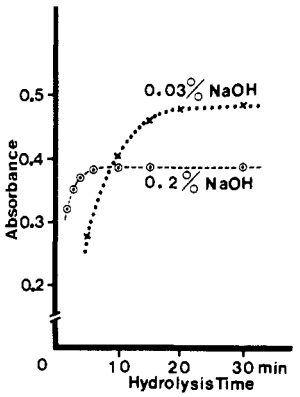

Figure 2. Absorbance at 580 nm of 50 μg of carbaryl in 20 mL of 0.03 and 0.2% NaOH in methanol solutions relative to hydrolysis time before adding 1 mL of NBDFB solution.

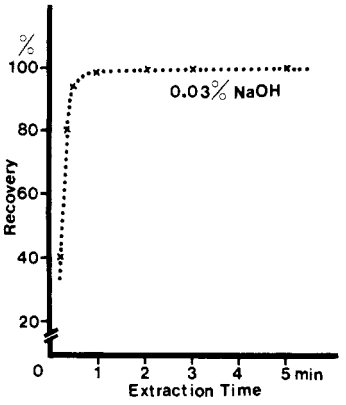

Figure 3. Percent recovery of 50 μg of carbaryl in 20 mL of 0.03% NaOH in methanol solution relative to extraction time.

sufficient time had elapsed after extraction for hydrolysis to be completed before adding the NBDFB solution.

Coefficients of variation (cv), obtained with 12 replicate runs with the complete range of standard solutions from 5 to 100 μg/20 mL, indicate that the error was substantially higher in the lower range. The cv's were 27 and 10% for 5 μg and 10 μg/20 mL, respectively; for 30 μg/20 mL and above the cv's remained less than 5%.

Carbaryl deposits were completely extracted in a period as short as 1 min (Figure 3). After 5-min extraction, the color developed was substantially darker to the naked eye, but little difference in the absorbance reading was observed. This deepening of color may be related to the chlorophyll component which has maximum absorbance near 390 nm but had little effect upon absorbance at 580 nm, the wavelength used. A 2-min extraction period proved to be optimal; it ensured extraction but did not interfere with color development. This extraction period was equally effective for fresh deposits and for those aged up to 21 days.

Concentration and volume of NBDFB solutions substantially influence the wavelength for maximum absorbance and color intensity. When an excess amount of NBDFB was used, the color changed to a reddish tone. The absorbance at 580 nm became much weaker with the excess NBDFB, and it had a direct connection with the alkaline concentrations as shown on Figure 4. In this method 1 mL of a solution containing 50 mg of NBDFB/100 mL of an acetone–methanol mixture (1:1) was found most suitable with 0.03% NaOH solution, but 0.2% solution was also a good choice as described previously. When pure CH_3OH was used to dissolve NBDFB, a very clear blue color was obtained, but NBDFB is difficult to dissolve in pure CH_3OH. Methanol is essential,

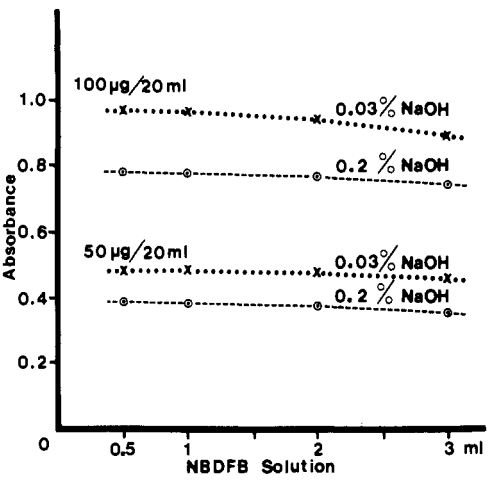

Figure 4. Absorbance at 580 nm of 50 and 100 μg of carbaryl in 20 mL of 0.03 and 0.2% NaOH in methanol solutions relative to quantities of NBDFB solution.

however, because acetone alone does not produce the desired color.

Under the conditions suggested, the spectrum of color ranged from red for a blank sample with the λ_{max} at 510 nm through purple for the 30 μg/20 mL sample with the λ_{max} at 545 nm to blue for the 100 μg/20 mL sample with the λ_{max} at 570 nm. When the λ_{max} was measured against the blank leaf extract as the reference, all the values fell within the range of 575–580 nm; the best linearity was obtained when absorbance was measured at 580 nm under the experimental conditions given. The developed color was very stable.

Test Tubes as Cuvettes. Disposable test tubes proved to be inexpensive and reliable cuvettes. Initially the absorbance recorded for 40 tubes ranged from 0.536 to 0.620 with the average of 0.580. After the tubes were marked and positioned accordingly, the readings were confined to a range of 0.568–0.582. The cv value for these recordings was 0.71%, which is superior to the value of 1.84% obtained with commercially available Hycel cuvettes.

Use of disposable tubes as received is probably acceptable if the purpose of the analysis is to check the spray coverage. Because, as demonstrated previously, spray coverage varies widely (Chiba, 1973; Chiba et al., 1973), 10% error in an absorbance reading does not seem critical. For example, deposits of Sevin within a tree ranged from 0.4 to 21.4 μg/cm^2 (Chiba, 1973) after spray application.

Further study proved that semiquantitative determination was possible by simple color matching with a series of standards which were prepared in test tubes. The use of a portable comparator (The Lovibond Comparator, made for the Tintometer Ltd., Salisbury, England) was also found to be a useful tool in the field.

Interference. Interference in color development is inevitable with this method if 1-naphthol is present. In this study, however, there was no indication of 1-naphthol on foliage of peach or grape up to 21 days after spraying. Likewise, Gunther et al. (1962) found no free 1-naphthol in lemons and oranges. Although it seems unlikely that any residues of 1-naphthol will be found on sprayed leaf samples, it may be a good practice to check for 1-naphthol occasionally.

None of the pesticides tested for possible interference produced a similar color when tested individually. Absorbance readings for tetradifon and folpet, however, increased ~20% when mixed with carbaryl.

Young leaves gave more color than old leaves regardless of the kind of foliage. However, essentially no interference

was observed when the suggested experimental procedures were followed.

Analytical Results. Values obtained from the individual analyses of 350 leaf disks, grouped in seven replicates of 50, ranged from 4.12 to 4.93 $\mu g/cm^2$; the overall average was 4.62 $\mu g/cm^2$. In contrast, three values obtained with the official AOAC method, where 50 disks constituted a single sample, were 4.05, 4.42, and 4.95, and the overall average was 4.47 $\mu g/cm^2$. These results validate the reliability of this rapid method. The significance of individual leaf analyses is well demonstrated in previous papers (Chiba, 1973; Chiba et al., 1973).

In this paper, the amount of carbaryl has been expressed as either $\mu g/cm^2$ of leaf surface or $\mu g/20$ mL of alkaline solution for convenience. If ppm values are necessary, conversion is simple; 1 $\mu g/cm^2$ is approximately equivalent to 100 $\mu g/g$ of leaf (Chiba and Northover, 1977).

CONCLUSION

The speed and simplicity of this method of analysis for carbaryl make it useful for studying spray distribution, fate of spray deposits, and wash effect of rainfall (Williams, 1961) on orchard trees. With it, an analyst can readily analyze 250 leaf disks in a day. Where a colorimeter or spectrophotometer is not available, semiquantitative determination is possible by color matching which is an added advantage. A portable comparator is also a useful tool in the field. Deposits on one side of leaf only can also be measured by wiping off the other side of the leaf before removing the disk (Herne and Chiba, 1975; Chiba et al., 1978). At present this method is only suitable for measuring carbaryl deposits on leaves and is not applicable for other materials such as foodstuffs.

ACKNOWLEDGMENT

The author thanks R. G. Richards and F. Doornbos for their valuable technical assistance.

LITERATURE CITED

Association of Official Agricultural Chemists, "Official Methods of Analysis", 10th ed.; AOAC: Washington, DC, 1965; p 407.
Chiba, M. *J. Econ. Entomol.* **1973**, *67*, 529.
Chiba, M.; Fisher, R. W.; Northover, J.; Herne, D. C.; Neff, A. *Can. J. Plant Sci.* **1973**, *53*, 189.
Chiba, M.; Morley, H. V. *J. Assoc. Off. Anal. Chem.* **1964**, *47*, 667.
Chiba, M.; Northover, J. *J. Agric. Food Chem.* **1977**, *25*, 39.
Chiba, M.; Phillips, J. H. H.; Roberts, M. D. *J. Econ. Entomol.* **1978**, *71*, 369.
Gordon, L.; Little, D. E. *Calif. Agric.* **1954**, *8*, (6).
Gunther, F. A.; Blinn, R. C.; Carman, G. E. *J. Agric. Food Chem.* **1962**, *10*, 222.
Gunther, F. A.; Ott, D. E. *Residue Rev.* **1966**, *14*, 12.
Herne, D. C.; Chiba, M. *Can. Entomol.* **1975**, *107*, 801.
Pielou, D. P.; Williams, K.; Brinton, F. E. *Nature (London)* **1962**, *195*, 256.
Williams, K. *Can. J. Plant Sci.* **1961**, *41*, 449.

Received for review May 8, 1980. Accepted September 22, 1980. Part of this paper was presented at the CIC/ACS Joint Conference, Toronto, Ontario, Canada, May 24–29, 1970.

Fate of *O*-[4-[(4-Chlorophenyl)thio]phenyl] *O*-Ethyl *S*-Propyl Phosphorothioate (RH-0994) in Cotton

Don L. Bull* and G. Wayne Ivie

After a single topical application to individual cotton leaves of field-grown cotton, residues of [^{14}C]RH-0994 [*O*-[4-[(4-chlorophenyl)thio]phenyl] *O*-ethyl *S*-propyl phosphorothioate] and its intact ester derivatives, either on the leaf surface or within the leaf, were essentially depleted at 14-days posttreatment. Studies of the distribution of radioactive residues in cotton plants after 10 spray applications of [^{14}C]RH-0994 [1.12 kg ha^{-1} (application)$^{-1}$] at 5-day intervals indicated that appreciable levels of radioactive material, including the parent compound and its intact ester derivatives, accumulated on foliage that was present during sprays. Mature cottonseed and lint from bolls that opened after treatments had been stopped also contained ~2 ppm of [^{14}C]RH-0994 equivalents; however, results of solvent extraction studies suggested that these residues did not include RH-0994 or its intact ester derivatives. Radioactive products of [^{14}C]RH-0994 identified in plants included the sulfoxide and sulfone derivatives of the intact ester, produced by oxidation of the thioether sulfur, and three substituted phenols, which were produced by hydrolysis of the respective esters and were present in both free and conjugated forms.

The experimental organophosphorus (OP) insecticide *O*-[4-[(4-chlorophenyl)thio]phenyl] *O*-ethyl *S*-propyl phosphorothioate (RH-0994, I) is being developed for possible use in controlling *Heliothis* spp. pests of cotton. Technical-grade RH-0994 is a dark amber-colored oil that is essentially insoluble in water but is soluble in most organic solvents. The acute oral toxicity (LD_{50}) to rats is 320 mg/kg and the acute dermal toxicity (LD_{50}) to rabbits is 1180 mg/kg (Hurt, 1980). This report describes studies of the fate of RH-0994 after application to cotton plants.

Cotton Insects Research Laboratory (D.L.B.) and Veterinary Toxicology and Entomology Research Laboratory (G.W.I.), Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, College Station, Texas 77841.

EXPERIMENTAL SECTION

Chemicals. [^{14}C]RH-0994 (I), formulated as an emulsifiable concentrate (4 EC, specific activity 3.23 mCi/g of active ingredient (AI); radiochemical purity of AI was 91%), was supplied by the Rohm and Haas Co., Spring House, PA. The molecule was uniformly radiolabeled in the *P*-*O*-phenyl moiety. Also provided were unlabeled, pure samples of technical-grade RH-0994 as well as samples of the potential metabolites: II, *O*-[4-[(4-chlorophenyl)sulfinyl]phenyl] *O*-ethyl *S*-propyl phosphorothioate; III, *O*-[4-[(4-chlorophenyl)sulfonyl]phenyl] *O*-ethyl *S*-propyl phosphorothioate; IV, 4-[(4-chlorophenyl)thio]-phenol; V, 4-[(4-chlorophenyl)sulfinyl]phenol; VI, 4-[(4-chlorophenyl)sulfonyl]phenol. Structures of these chem-