The Use of Electron Beam Analysis to Determine the Deposition of Chlorothalonil Smoke Particles in a Greenhouse[1]

C. Tappan[2], C.R. Krause[2], and C.C. Powell, Jr.[3]
U.S. Department of Agriculture, Agricultural Research Service
Application Technology Research Unit, OARDC, Wooster OH 44691

Abstract

Electron beam analysis (EBA) was used to measure chlorothalonil smoke particles on artificial target surfaces in greenhouses. Particles between 0.4 μm (0.000016 in) and 3.0 μm in diameter were found to vary significantly in number with location within a poinsettia canopy and with distance from the source of the smoke. Particles did not vary significantly in size either within the canopy or with distance from the source. No measurable residue was found when the greenhouse was not tightly sealed. EBA proved to be a viable method of investigating fungicide smoke deposition and can provide precise information about the environmental fate of pesticides related to application technology.

Index words: scanning electron microscopy, energy dispersive X-ray analysis, digitized images, Exotherm Termil fungicide.

Significance to the Nursery Industry

This research was intended to help answer the question, "How can pesticide usage be reduced while still providing effective treatment?". An even distribution of small fungicide particles throughout the plant canopy is generally the desired result, yet there is really no adequate method of determining spatial distribution. If there were an effective method of determining spatial distribution of various sized particles, research could be undertaken to improve formulations and delivery systems.

Introduction

Ultra-low volume (ULV) pesticide delivery systems such as smokes, fogs, and aerosol generators have been developed to reduce the cost of pesticide applications and to prevent potential environmental hazards in greenhouses (7, 9, 10).

The efficacy of a pesticide is largely dependent on its distribution onto target surfaces. Efficacy generally improves as particle size decreases (1, 2, 3, 8), provided the particles distribute evenly onto the target surfaces. Very small particles are subject to many influences, such as drift, sublimation, and poor penetrability in static air. These influences work against even and efficacious distribution of the active ingredient. As particle size decreases, current research methodology becomes inadequate for discerning the efficacy, behavior, or fate of pesticides used in combination with various ULV delivery systems (5, 11). Chemical residue bioassays do not reveal particle size and spatial patterns. Light microscopy does not positively detect small particles due to the limits of the resolving power of visible light (approximately 10 μm). The lack of positive particle identification results in a lack of information about pesticide residue distribution. A lack of such information precludes analyses which might optimize dosage.

Electron beam analysis (EBA) provides a method of measuring pesticide deposition, especially when particles are too small to be easily seen with light microscopy. EBA, a combination scanning electron microscope (SEM) energy dispersive X-ray analysis (EDXA) and digital image analysis, provides a method of directly relating a pesticide application to resulting particle distribution on target and non-target surfaces (12). Krause and Powell (5) have used EBA to characterize vinclozolin particles emitted from self-dispersing smoke generators. Particles may be typed by size, shape, and elemental composition (4, 6, 12).

This study is a continuation of research reported by Krause and Powell (5) to further develop EBA metholology for tracking fungicide deposition and distribution patterns produced by self-dispersing smoke generators in greenhouse environments. In the first part of the study, deposition of fungicide particles was measured in relation to location within a typical greenhouse crop canopy. In the second series of experiments, fungicide particle size and quantity were measured in relation to distance from the smoke generator in a greenhouse.

Materials and Methods

Placement of pesticide sample collection mounts: Poinsettia (*Euphorbia pulcherima* 'Brilliant Diamond') plants, 45 cm (17.6 in) tall and rooted in 22 cm (8.6 in) pots, were used in the first part of the study to provide canopy level deposition data. Studies were conducted in a 30 m^2 greenhouse. Aluminum mounts, 7.5 mm (0.3 in) in diameter (Ted Pella, Redding, CA), were used as inert, pesticide residue sample collection surfaces and were coated with colloidal graphite in isopropanol. These mounts were placed 2 m (6.6 ft) from the source of the smoke at the bottom of the canopy (at soil level) and at the top of the canopy (on stands 65 cm (25.4 in) from the bench surface). Mounts were stored in airtight plastic boxes (Ted Pella, Redding, CA) before and after applications. In the second part of the study, no plant mate-

[1]Received for publication January 16, 1996; in revised form October 7, 1996. In partial fulfillment of a Masters of Science degree from The Ohio State University by the senior author. Supported in part by grants from **The Horticultural Research Institute, 1250 I Street, N.W., Suite 500, Washington, D.C. 20005** and the National Pesticide Impact Assessment Program. The authors wish to express their appreciation to Ms. Leona E. Horst and Mr. Matthew S. Krause for their technical assistance and to Mr. Justin Marotta, Possum Run Greenhouse, Bellville, OH.

[2]Plant pathologists.

[3]Plant Health Advisory Services, Worthington, OH.

rial was used. Mounts were placed at distances of 0.6, 4.0, 5.5, and 9.0 m (2.1 ft, 13.2 ft, 18 ft, and 29.7 ft respectively) from the smoke source, 22 cm (8.6 in) above ground level in a 50 m^2 (540 ft^2) greenhouse. An adjacent 30 m^2 (324 ft^2) greenhouse was used to provide a control (non-fumigated) environment.

Fungicide treatment: The treated greenhouses were exposed overnight (16 h) to Exotherm Termil[(R)] (Rigo Chemical Co., Buckner, KY), the self dispersing smoke form of chlorothalonil or 2,4,5,6-tetrachloroisophthalonitrile (TCIN), ISK Biosciences Corp., Mentor, OH, in 100 g (3.5 oz) canisters. The manufacturer's printed instructions were followed

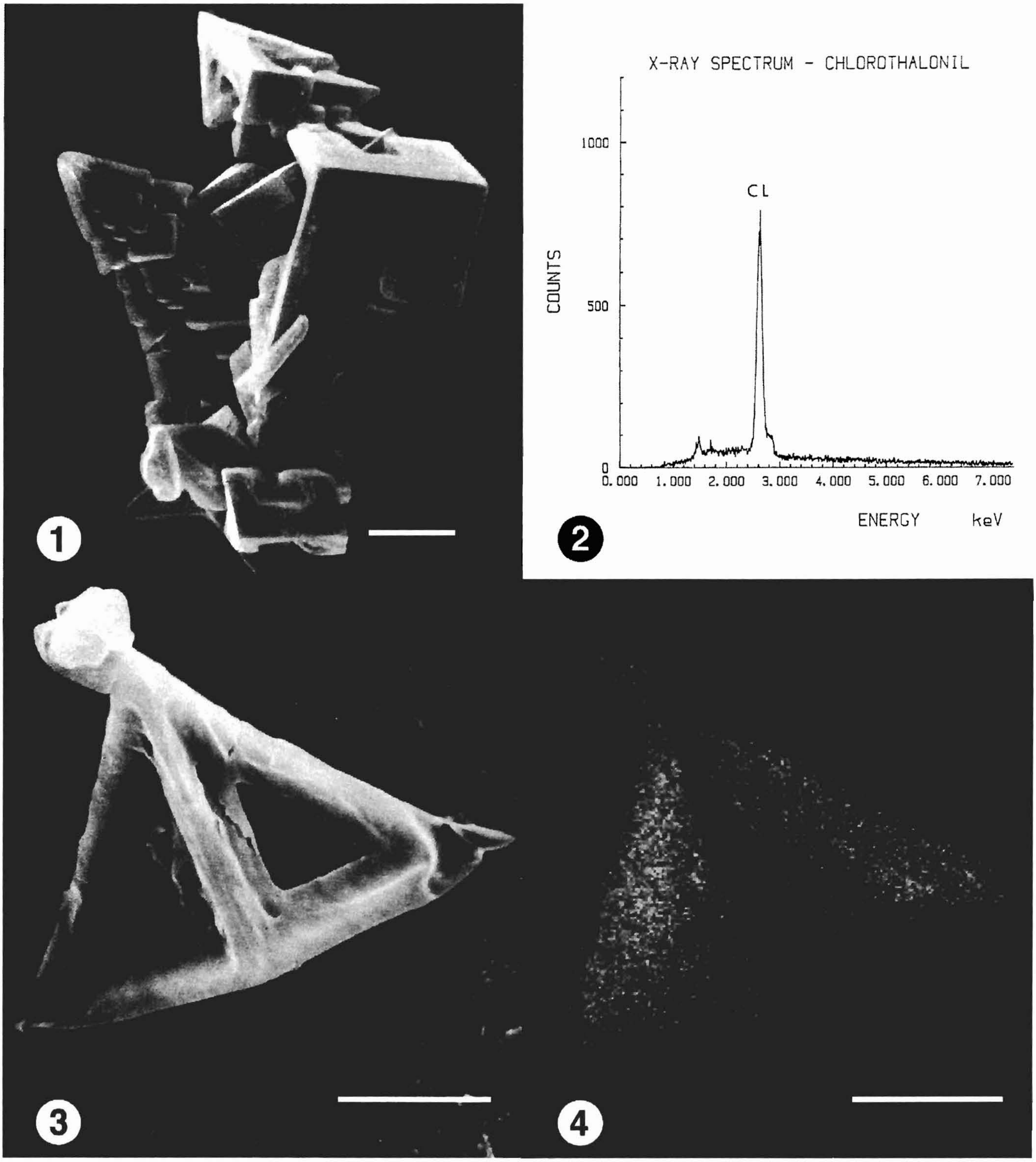

Fig 1. 1) Visual image of typical TCIN technical grade particle (Bar = 20 μm). 2) X-ray line spectrum of TCIN technical grade particle shown in 1, showing large chlorine peak. 3) Visual image of typical formulated Exotherm Termil[(R)] residue particle. 4) X-ray dot map of particle shown in 4, showing elemental chlorine concentration (Bars = 4 μm).

Table 1. **Mean number of TCIN particles found per 2.5×10^4 m² in the upper and lower poinsettia canopy.**

	Mean (no.)		
Canopy level	Expt. 1	Expt. 2	Expt. 3
Upper	6.8 (4)[z]	16.3 (8)	7.9 (10)
Lower	4.8 (5)	8.7 (12)	3.9 (8)
LSD (0.02)[y]	1.1	7.0	2.3

[z]Replications, each the sum of 10 subsamples. Each subsample area = 2500 μm².

[y] Within each experiment, all means are significantly different at t = 0.02.

Table 2. **Mean diameter (μm) of TCIN particles found in the upper and lower canopy poinsettia canopy.**

	Diameter (μm)		
Canopy level	Expt. 1	Expt. 2	Expt. 3
Upper	4.48 (30)[z]	3.86 (40)	2.85 (29)
Lower	4.39 (23)	2.92 (30)	2.12 (15)
LSD (0.05)[y]	0.90	0.96	1.29

[z]Number of particles measured.

[y]No significance between means at t = 0.05

regarding dosage and time of exposure. The greenhouses were vented for 15 minutes before retrieving the mounts.

Microscopy and microanalysis: All specimen mounts were prepared for examination by carbon coating them in a vacuum evaporator (Mikros Inc. Model VE 10, Portland, OR). Mounts were examined on a rotating, tilting stage within a Hitachi S-500 scanning electron microscope (SEM) (Nissei Sangyo America LTD, Mountain View, CA). The SEM was equipped with an EDXA (Model 5502; Noran Inc., Middleton, WI), employing an identification program and digital beam control hardware with X-ray mapping programs (MSCAN II; Noran, Inc.). Mounts were examined at 15 mm (0.6 in) working distance with a 20 kV accelerating current. Each replication in the measurement of mean number of TCIN particles is the sum of ten randomly selected subsample areas from a single mount. In the measurement of mean diameter of TCIN particles, a random number of particles was obtained, sized, and chemically typed through computer software (PRC; Noran, Inc.) attached to the SEM. Experimental means were compared using Student's t-test.

Technical grade TCIN (unfomulated) was obtained from the manufacturer for comparison with particles found on the mounts in order to confirm size range, shape and elemental composition of the experimental commercial material. The technical grade material was mounted and observed under identical sample preparation as for the experimental material.

Results and Discussion

Electron beam analysis of the technical grade material and TCIN-treated mounts revealed complex crystalline particles in a range of 0.4 to 90.0 μm in size (Fig. 1–1) not found in controls. Corresponding X-ray line spectra included large chlorine peaks (Fig. 1–2) always associated with these crystals. EBA also yielded images of crystals with corresponding chlorine X-ray dot maps (Figs. 1–3, 1–4). Positive identification of the size range, shape, and elemental composition of technical grade TCIN particles allowed for rapid data collection from the treated mounts through the computer software attached to the SEM.

The mean numbers of TCIN particles found on specimen mounts in the upper and lower poinsettia canopy are shown in Table 1. The number of particles was found to be 42% to 103% greater in the upper canopy.

The mean diameters of particles found on mounts in the upper and lower canopy are shown in Table 2. The lack of any significant difference in particle size between the upper and lower canopy indicates that although roughly double the amount of mass was deposited in the upper canopy, all of the increase was a result of the number of particles rather than larger sized particles.

Table 3 shows the number of particles as a function of distance from the source of the fumigant, the subject of the second part of the study. The smaller number of particles found at 0.6 m (2 ft) could have been a result of a convection current flowing upward at the heat source. Moving air at this point might impede deposition. Temperature patterns were not measured. The smaller number of particles at 9.0 m (29.7 ft) could be a function of air turbulence along the greenhouse walls.

Average particle size did not vary with distance, as shown in Table 4. One can estimate that between 50% to 100% more deposition occurred from 4.0 and 5.5 m (13.2 and 18.2 ft)

Table 3. **Mean number of TCIN particles per 6300 μm² found at varying distances from the source of the smoke.**

	Mean[z] (no.)	
Distance (m)	Expt. 1	Expt. 2
0.6	29.0a (4)[y]	26.0a (4)
4.0	40.0b (6)	40.8b (6)
5.5	38.5b (4)	30.3c (4)
9.0	20.4c (8)	22.5a (8)

[z]Means followed by common letters are not significantly different at t = 0.05. Experiments were analyzed separately.

[y]Replications, each the sum of 10 subsamples, each subsample equal to 630 μm².

Table 4. Mean diameter (μm) of TCIN particles found at varying distances from the source of the smoke.

	Diameter[z] (μm)	
Distance (m)	Expt. 1	Expt. 2
0.6	2.36 (32)[y]	2.61 (32)
4.0	2.68 (48)	2.57 (48)
5.5	2.21 (32)	2.00 (32)
9.0	2.27 (64)	2.25 (64)

[z]No significant difference between distance means at t = 0.05. Experiments were analyzed separately.

[y]Number of particles measured.

compared to 0.6 and 9.0 m (2 and 29.7 ft) due to the number of particles deposited, and not their size.

The reason(s) for the difference between mean particle diameters in the canopy penetration experiments (2.12 to 4.48 μm) compared to the greenhouse spatial distribution experiments (2.00 to 2.68 μm) is noteworthy. Environmental variables such as temperature or humidity could explain these differences. Another cause could have been formulation differences. Further research as to the cause and significance of these particle size differences is needed.

In these experiments, EBA proved to be a viable method of investigating pesticide particle distribution via smoke application. Particles smaller than 1 μm (0.000039 in) were positively identified as TCIN visually as well as by elemental analysis. The latter could not be achieved by light microscopy.

The question of dosage and its relationship to efficacy should be addressed in future research by correlating EBA residue data with that of conventional analytical chemical methods. The size and distribution of particles on the target surface has an important bearing on efficacy (3, 8). These data cannot be obtained by chemical analysis. Residue mass of a specific pesticide could be determined by volumetric analysis of a sample area and these data then correlated with data obtained by chemical analysis. On this basis, EBA could then be used both as a quantitative and qualitative methodology in efficacy studies.

General consistency of data among replications and experiments in this study suggests that, in conjunction with a quantitative technique, EBA could potentially provide more precise information relative to environmental fate and behavior of pesticides. Such data is useful to improve efficacy, for development of worker safety standards, to enhance water quality and for reduction of pesticide usage.

Literature Cited

1. Balls, E.J. 1978. Reduction of active ingredient dosage by selecting appropriate droplet size for the target. Brit. Crop Prot. Conf.—Symposium on Controlled Drop Application held 12–13 April 1978. University of Reading. p. 101–106.

2. Himel, C.M. and A.D. Moore. 1969. Spray droplet size in the control of spruce budworm, boll weevil, bollworm, and cabbage looper. J. Econ. Ent. 62, 4:916–918.

3. Himel, C.M. 1969. The optimum size for insecticide spray droplets. J. Econ. Ent. 62, 4:919–925.

4. Krause, C.R. 1985. Foliar penetration of a fungicide as detected by scanning electron microscopy and energy dispersive X-ray analysis. Scanning Electron Microscopy 11:811–815.

5. Krause, C.R. and C.C.Powell. 1988. Measurement of fungicide smoke application by electron beam analysis. Scanning Microscopy 3, 1:243–248.

6. Levin, E.R. 1986. Principles of X-ray spectroscopy. Scanning Electron Microscopy 1:65–76.

7. Matthews, G.A. 1982. New developments in pesticide application technology. Crop Protection 1, 2:131–145.

8. Mboob, S.S. 1975. Preliminary assessment of the effectiveness of two droplet sizes of insecticide for the control of greenhouse whitefly. Plant Pathology 24:56–65.

9. Owens, J.M. and G.W. Bennett. 1977. Ultra-low-volume spraying in the greenhouse. Florist's Review. Oct. 20 40–53.

10. Powell, C.C., R.K. Lindquist, and H.J. Retzer. 1983. The use of thermal pulse-jet applicators in greenhouses. Ohio Report, May-June 35–37.

11. Uk, S. 1977. Droplets in air, Part 2: Capture of natural surfaces. Pest. Sci. 8:501–509.

12. Zeyen, R.J. 1982. Applications of in situ microanalysis in understanding disease: X-ray microanalysis. Ann. Rev. Phytopathology 20:119–142.