Residue Levels, Decline Curves, and Plantation Distribution of Procymidone in Green Beans Grown in Greenhouse

José Castillo-Sánchez, Ana Aguilera-del Real, Mariano Rodriguez-Sánchez, and Antonio Valverde-García*

Pesticide Residue Research Group, Facultad de Ciencias Experimentales, Universidad de Almería 04071 Almería, Spain

Residue levels and degradation rates of procymidone residues were studied in green beans grown in a greenhouse. Experiments were planned to also assess the influence of planting density on the behavior of procymidone residues on this type of crop. The study was carried out in four random blocks considering three sub-blocks of different planting densities into each block. Plants were sprayed with Sumisclex 50 WP (1077.5 g of ai/ha) 52 days after the transplantation, and sampling was carried out daily during two different periods of 6 and 5 consecutive days, respectively, around the two harvest days (days 12 and 28 after the treatment). Residue levels of procymidone were determined by using the Luke extraction method and GC-NPD. The average residue levels of procymidone in the overall planting (mean of 12 determinations) were below 2 mg/kg (European maximum residue limit) for all the sampling days, obtaining values of 1.01 ± 0.55 and 0.37 ± 0.10 mg/kg, respectively, at the two harvest days. The decline behavior of procymidone residues in the overall plantation and in each block could be described as a pseudo-first-order reaction, obtaining half-life values ($t_{1/2}$) of 10−11 days in all cases. The calculated residue level at the preharvest time (5 days) in the overall plantation was 1.7 mg/kg, but this value in the blocks depended on the block position along the greenhouse and ranged from 2.3 to 0.9 mg/kg. In this work, additional data on the residual behavior of the fungicide pyrazophos in green beans were also obtained.

Keywords: *Procymidone; pyrazophos; residues; green beans*

INTRODUCTION

Procymidone is a dicarboxamide fungicide effective for the control of *Botrytis*, *Sclerotinia*, *Monilia*, and *Helminthosporium* species on fruits, vines, vegetables, cereals, and many other crops. It is a systemic fungicide with protective and curative properties, which was introduced by Sumitomo Chemical Co. with the tradenames of Sumisclex and Sumilex (Tomlin, 1994). Procymidone is widely used all over the world, and its residues are commonly found by regulatory agencies of many countries in their pesticide residue monitoring (FDA, 1993; Andersson et al., 1998; MAPA, 1998).

Residues of procymidone in foods were evaluated by the FAO/WHO Expert Groups on Pesticide Residues in 1989 (FAO, 1990). The behavior of procymidone residues has been studied on different greenhouse and field-grown fruits and vegetables, but most of the data published in the open literature are focused on tomatoes and grapes (Cabras et al., 1985; Apladasarlis et al., 1994; Garcia-Cazorla and Xirau-Vayreda, 1994). Some data on the behavior of procymidone residues in different fruits processing have also been recently published (Sala et al., 1996; Cabras et al., 1998a−c).

The objectives of this work were to evaluate the degradation rate and residue levels of procymidone in green beans grown in a plastic greenhouse using the typical horticultural practices and particular climatic conditions of this type of plantation. An additional objective initially established in this work was to assess the influence of the planting density on the behavior of procymidone residues in greenhouse-grown green beans.

* To whom correspondence should be addressed (telephone: +34 950 215309; fax: +34 950 215070; e-mail: avalverd@ualm.es).

EXPERIMENTAL PROCEDURES

Chemicals and Apparatus. Standards of procymidone and pyrazophos were supplied by Riedel-de Haën (Seelze, Germany). All solvents (pesticide residue grade) were obtained from Merck (Darmstadt, Germany).

The gas chromatograph was a Hewlett-Packard model 5890 equipped with a HP-1 wide-bore fused capillary column (10 m × 0.53 mm i.d. × 2.65 μm film thickness) attached to a nitrogen−phosphorus detector (NPD). The chromatographic conditions were detector temperature 270 °C; injector temperature 240 °C; oven temperature program, 1 min at 200 °C, 30 °C/min to 230 °C and hold for 7 min; carrier gas (nitrogen) flow rate 14 mL/min; air flow rate 115 mL/min; hydrogen flow rate 3 mL/min; and injection volume 2 μL. The retention times of procymidone and pyrazophos in this column and GC conditions were 2.25 and 6.33 min, respectively.

Extraction Procedure. Extraction of procymidone and pyrazophos residues in green bean samples was carried out according to a modification of the Luke extraction procedure (Luke et al., 1975; Valverde and González, 1989). A brief description of the procedure is as follows: weigh 50 g of chopped sample into a high-speed blender jar, add 100 mL of acetone, blend the mixture for 2 min, and filter through a 12-cm Büchner funnel; transfer the filtrate into a 1000-mL separatory funnel, add 200 mL of *n*-hexane:Cl_2CH_2 (1:1) mixture, and shake vigorously for 1 min; transfer the aqueous layer (lower) to another separatory funnel, add 15 mL of saturated NaCl solution and 70 mL of CH_2Cl_2, and shake for 1 min; wash the aqueous layer (upper) with another 70 mL of CH_2Cl_2 and discard the aqueous layer; filter all the organic

10.1021/jf990770y CCC: $19.00
Published on Web 06/24/2000

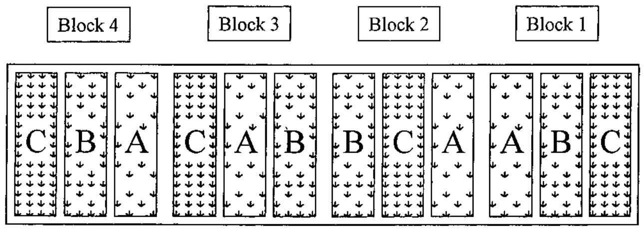

Figure 1. Distribution of the green beans plantation.

portions through anhydrous sodium sulfate and evaporate the sample extract to 1 mL, aproximately, using a vaccum rotatory evaporator (40 °C water bath); and finally, add 10 mL of *n*-hexane, evaporate again to 1 mL, and dilute to exactly 10 mL with a mixture of *n*-hexane:acetone (9:1). In all instances, it was assumed that this final extract represented 5 g of sample/mL. Procymidone and pyrazophos residues contained in these extracts were determined by GC-NPD using the operating conditions described above. Mean recoveries obtained with this method on blank green bean samples spiked with procymidone at the levels of 0.28, 0.56, 1.41, and 2.82 mg/kg ranged between 89 and 93%, with standard deviations less than 7% (three replicates for each spiking level). Mean recoveries obtained for pyrazophos at the spiking levels of 0.10, 0.20, and 1.00 mg/kg were 99, 101, and 93%, respectively, with standard deviations less than 6% in all cases.

Decline Study. Decline experiments were conducted in an experimental greenhouse located at the Agricultural Research Centre "Las Palmerillas" in Almería, Spain. The study was carried out in four random blocks distributed longitudinally along the half-south plot (49 × 13 m) of an experimental greenhouse of 1274 m^2, considering three sub-blocks of varying planting densities in each block: density A (15 plants/m^2), density B (21 plants/m^2), and density C (27 plants/m^2). Sub-blocks consisted of four plantation rows spaced at 1 m, each row containing 30 plantation points spaced at 0.33 m. Five, seven, and nine plants (variety Strike) were planted in each plantation point in sub-blocks A, B, and C, respectively. The global distribution of the plantation is shown in Figure 1.

Green bean plants, receiving routine horticultural treatment, were sprayed (52 days after planting) with Sumisclex 50 WP (procymidone 50%) at a dose of 0.1% and at an application rate of 1077.5 g of ai/ha. Previously, the plants were treated two times (22 and 46 days after planting) with Afugan (pyrazophos 30% w/v) at application rates of 750 and 1400 g of ai/ha, respectively.

Following the usual horticultural procedures for this type of crop, the harvest was carried out in two phases: the first one, 64 days after planting and the last one, 79 days after planting. Sampling was predetermined to occur during two different periods of 6 and 5 consecutive days, respectively, around the two harvest days. Specifically, samples were collected at 9, 10, 11, 12 (first harvest), 13, 14, 24, 25, 26, 27, and 28 (last harvest) days after procymidone application. In each sub-block, two samples of approximately 200 g each were taken every sampling day from different rows (rows 1 and 3 on odd sampling days and rows 2 and 4 on even sampling days). After picking, the samples were put into polyethylene bags and transported immediately to the laboratory. Analytical samples were obtained by mixing the two replicate samples from each sub-block. These samples (12 per sampling day) were chopped, thoroughly mixed, and kept deep-frozen until analysis. Residue levels of procymidone and pyrazophos were determined by applying the extraction and GC methods described above. In all cases, no more than 2 h passed from sampling to storage in the freezer, analyses being carried out between 24 and 48 h after samples were stored in the freezer. Stability of procymidone and pyrazophos in green beans during the homogenization and storage procedures were previously tested on spiked samples.

The average daily maximum/minimum temperatures outside and inside the greenhouse throughout the study were 26/8 °C and 30/7 °C, respectively, whereas the maximum/minimum absolute temperatures outside and inside the greenhouse were 33/5 °C and 36/4.5 °C, respectively. Average maximum/minimum relative humidity outside and inside the greenhouse were 97/46% and 97/43%, respectively, and maximum and minimum solar irradiation outside the greenhouse were 5178 and 1520 W h/m^2 day, respectively.

RESULTS AND DISCUSSION

Procymidone residue levels determined every sampling day in each sub-block of the plantation are indicated in Table 1. The average residue levels of procymidone obtained every sampling day in the overall plantation (mean of 12 determinations) and in each block (mean of three determinations) are shown in Table 2. The average residue levels of procymidone in the overall plantation were, throughout the study, below 2 mg/kg (European MRL), and values of 1.01 ± 0.55 and 0.37 ± 0.10 mg/kg, respectively, were obtained at the two harvest days. However, during the first sampling period, six samples from block 1 were determined to contain procymidone levels between 2.03 and 2.66 mg/kg.

Average residue data in the overall plantation and in each block were subjected to statistical analysis to evaluate the decline of procymidone residues as a function of time and to determine the parameters that describe this process. Statistical analysis was carried out according to the formal approach proposed by Timme and Frehse (1980) to study the behavior of pesticide residues on crops prior to harvest. This approach assumes that decline the behavior of pesticide residues can be described as a pseudo-first-order reaction and quantified by linear semilogarithmic regression

Table 1. Residue Level of Procymidone for Each Sampling Day in Each Block and Sub-Block of the Plantation

	residue level, mg/kg											
time	block 1			block 2			block 3			block 4		
(days)[a]	A	B	C	A	B	C	A	B	C	A	B	C
9	1.40	2.03	2.66	1.38	1.87	1.11	0.90	1.75	0.76	1.37	1.52	0.25
10	0.98	2.30	2.04	0.88	1.29	1.04	0.87	0.76	1.13	1.18	0.91	0.24
11	0.99	1.78	2.44	0.64	0.62	1.62	1.24	1.55	1.46	0.66	0.79	0.07
12[b]	1.22	0.61	1.69	1.36	1.55	1.42	0.53	0.83	1.43	1.27	0.15	0.09
13	1.06	2.11	0.55	0.47	1.22	0.86	0.94	1.01	0.52	0.24	0.62	0.54
14	0.80	1.57	1.68	1.12	0.65	0.87	0.45	0.40	0.68	0.46	0.86	0.11
24	0.59	0.78	1.06	0.51	0.59	0.51	0.42	0.49	0.59	0.33	0.19	0.07
25	0.98	0.39	0.57	0.34	0.48	0.59	0.44	0.49	0.45	0.49	0.32	0.06
26	0.59	0.44	1.04	0.40	0.66	0.53	0.34	0.40	0.32	0.27	0.21	0.24
27	1.02	0.33	0.28	0.39	0.35	0.55	0.25	0.18	0.30	0.35	0.25	0.10
28[c]	0.52	0.35	0.60	0.35	0.41	0.34	0.35	0.23	0.36	0.26	0.38	0.30

[a] Days after procymidone treatment. [b] First harvest. [c] Second harvest.

Table 2. Average Residue Levels of Procymidone and Standard Deviations (SD) for Each Sampling Day in the Overall Plantation and in Each Block

time (days)[a]	residue level,[b] mg/kg (SD)				
	overall plantation	block 1	block 2	block 3	block 4
9	1.42 (0.63)	2.03 (0.63)	1.45 (0.39)	1.14 (0.54)	1.05 (0.69)
10	1.14 (0.55)	1.77 (0.70)	1.07 (0.21)	0.92 (0.19)	0.78 (0.48)
11	1.16 (0.65)	1.74 (0.73)	0.96 (0.57)	1.42 (0.16)	0.51 (0.38)
12[c]	1.01 (0.55)	1.17 (0.54)	1.44 (0.10)	0.93 (0.46)	0.50 (0.66)
13	0.85 (0.49)	1.24 (0.80)	0.85 (0.38)	0.82 (0.27)	0.47 (0.20)
14	0.80 (0.47)	1.35 (0.48)	0.88 (0.24)	0.51 (0.15)	0.48 (0.38)
24	0.51 (0.26)	0.81 (0.24)	0.54 (0.05)	0.50 (0.09)	0.20 (0.13)
25	0.47 (0.21)	0.65 (0.30)	0.47 (0.13)	0.46 (0.03)	0.29 (0.22)
26	0.45 (0.23)	0.69 (0.31)	0.53 (0.13)	0.35 (0.04)	0.24 (0.03)
27	0.36 (0.24)	0.54 (0.41)	0.43 (0.11)	0.24 (0.06)	0.23 (0.13)
28[d]	0.37 (0.10)	0.49 (0.13)	0.37 (0.04)	0.31 (0.07)	0.31 (0.06)

[a] Days after procymidone treatment. [b] Average of 12 determinations (overall plantation) or 3 determinations (blocks). [c] First harvest. [d] Second harvest.

Table 3. Statistical Quantities and Decline Parameters Corresponding to the Decline Study of Procymidone in Green Beans

	r^2	D	$t_{1/2}$ [CI][a] (days)	R_0 [CI] (mg/kg)	R_5 [CI] (mg/kg)
overall plantation	0.971	0.384	10.9 [± 1.4]	2.19 [+0.38/−0.33]	1.59 [+0.21/−0.18]
block 1	0.942	0.368	11.0 [± 2.1]	3.18 [+0.83/−0.66]	2.32 [+0.45/−0.38]
block 2	0.909	0.351	11.6 [± 2.8]	2.16 [+0.69/−0.52]	1.60 [+0.38/−0.31]
block 3	0.837	0.313	10.2 [± 3.4]	2.05 [+1.13/−0.74]	1.46 [+0.59/−0.42]
block 4	0.807	0.296	11.4 [± 4.2]	1.21 [+0.67/−0.43]	0.89 [+0.36/−0.25]

[a] CI, confidence interval at a significance level of $\alpha = 0.05$.

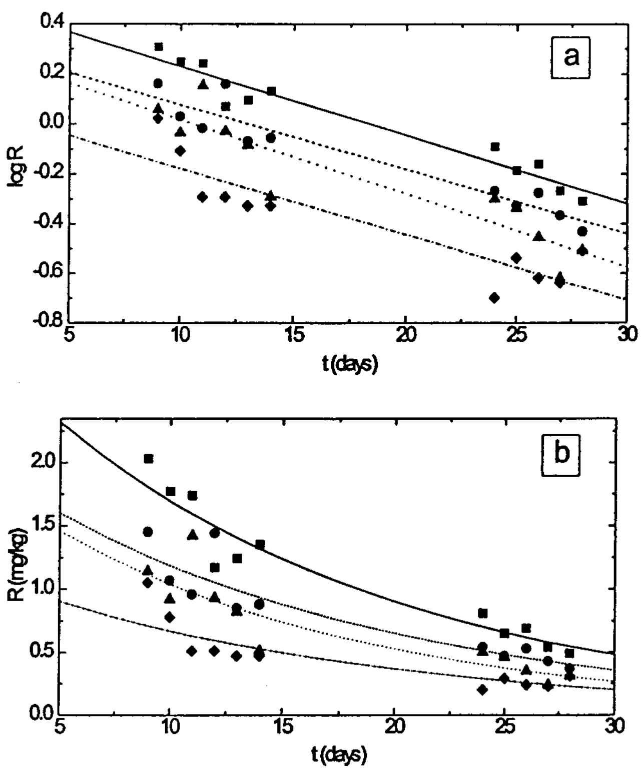

Figure 2. (a) Straight lines and (b) decline curves (back-transformed functions) obtained for procymidone residue data in blocks 1 (■), 2 (●), 3 (▲), and 4 (◆) by applying the first-order reaction model.

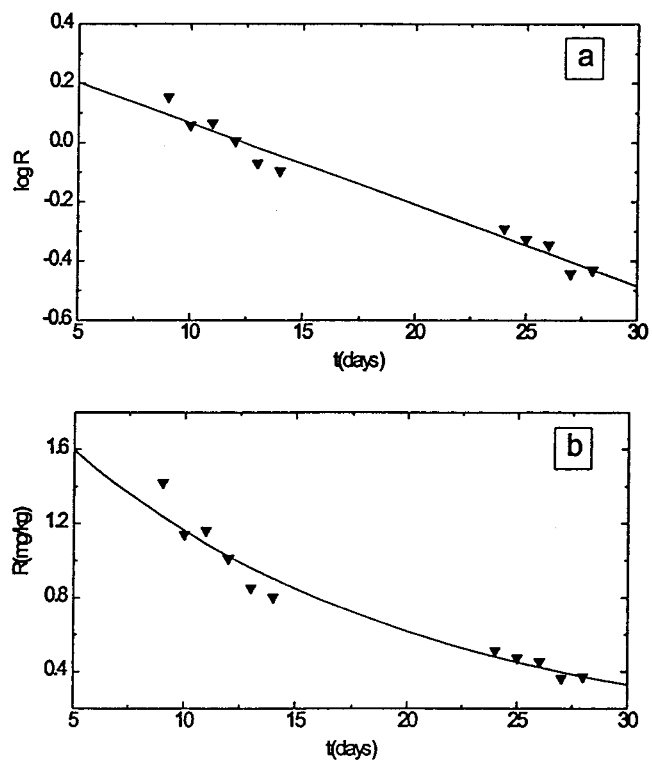

Figure 3. (a) Straight lines and (b) decline curves (back-transformed functions) obtained for procymidone residue data in the overall plantation by applying the first-order reaction model.

analysis. According to the above, the logarithms of residue values in Table 2 were plotted versus time, and the straight lines that best fit the measured values were computed by regression analysis. Figures 2a and 3a show the straight lines obtained for each block and the overall plantation, respectively. The corresponding decline curves are plotted in the original system (back-transformed function) in Figures 2b and 3b. In Table 3, calculated values of r^2 (coefficient of determination) and D (test quantity for correlation) are shown for the overall plantation and each block. In all cases, D values were greater than zero, which confirmed that the decline behavior of procymidone residues in green beans can be described as a pseudo-first-order reaction (Timme and Frehse, 1980). Procymidone half-life times ($t_{1/2}$), initial residues (R_0), and residues at preharvest time (R_5), determined from the first-order reaction model, are also given in Table 3.

Procymidone residue levels in the overall plantation after expiration of the preharvest interval of 5 days (Liñán y Vicente, 1999) was 1.6 mg/kg, which represents 80% of the MRL. However, the R_5 values obtained in the four blocks of the plantation were significantly different and varied from 2.4 mg/kg for block 1 to 0.9 mg/kg for block 4. These results, together with the R_0 values obtained in each block, confirmed that the distribution of procymidone in the plantation was not uniform and demonstrate that routine pesticide treatments, including when they are made by qualified personnel, can lead to a "lot" of a produce in which the homogeneity, in terms of pesticide residues content, is not guaranteed.

The half-life obtained for procymidone in the green bean plantation was 10.9 $\pm$ 1.4 days, the $t_{1/2}$ values obtained in each block being very similar. This half-life value is practically the same as that obtained by Cabras et al. (1985) for procymidone in greenhouse-grown tomatoes.

Because procymidone residue levels in the green bean samples was dependent on the block position in the greenhouse, it was assumed that the possible influence of the planting density on the behavior of procymidone residues in this type of crop could not be demonstrated. The results obtained in the application of an analysis of variance (ANOVA) to the data given in Table 1 confirmed this assumption.

Finally, the maximum and minimum residue levels of pyrazofos determined in the green bean samples analyzed throughout the study were 0.09 mg/kg and 0.02 mg/kg, respectively. The average residue level of pyrazofos in the overall plantation decreased from 0.06 (first sampling day: 16 days after the second treatment with pyrazophos) to 0.02 mg/kg (last sampling day: 35 days after the second treatment with pyrazophos). These results are in agreement with the maximum residue limit (0.1 mg/kg) admitted in Spain for pyrazophos in green beans and indicate that the preharvest interval of 15 days currently established in Spain for this systemic fungicide (Liñan y Vicente, 1999) seems to be adequate.

ACKNOWLEDGMENT

The authors thank Jose López-Gálvez and Francisco Bretones (Agricultural Research Centre "Las Palmerillas-Caja Rural de Almeria") for their collaboration in this work.

LITERATURE CITED

Andersson, A.; Palsheden, H.; Bergh, T.; Jansson, A. Pesticide Residues in Food of Plant Origin 1996. *Swedish National Food Administration Report 25*; 1997.

Apladasarlis, P.; Liapis, K. S.; Miliadis, G. D. Study of Procymidone and Propargite Residue Levels Resulting from Application to Greenhouse Tomatoes. *J. Agric. Food Chem.* **1994**, *42*, 1575−1577.

Cabras, P.; Meloni, M.; Pirisi, F. M.; Cabitza, F. Behavior of Acylanilide and Dicarboximidic Fungicide residues on Greenhouse Tomatoes. *J. Agric. Food Chem.* **1985**, *33*, 86−89.

Cabras, P.; Angioni, A.; Garau, V. L.; Melis, M.; Pirisi, F. M.; Cabitza, F.; Cubeddu, M. Pesticide Residues on Field-Sprayed Apricots and in Apricot Drying Processes. *J. Agric. Food Chem.* **1998a**, *46*, 2306−2308.

Cabras, P.; Angioni, A.; Garau, V. L.; Melis, M.; Pirisi, F. M.; Cabitza, F.; Pala, M. Pesticide Residues in Raising Processing. *J. Agric. Food Chem.* **1998b**, *46*, 2309−2311.

Cabras, P.; Angioni, A.; Garau, V. L.; Pirisi, F. M.; Brandolini, V.; Cabitza, F.; Cubeddu, M. Pesticide Residues in Prune Processing. *J. Agric. Food Chem.* **1998c**, *46*, 3772−3774.

FAO. Pesticide Residues in Food - 1989, FAO Plant Production and Protection Paper 99; Food and Agricultural Organization: Rome, 1990.

Food and Drug Administration. FDA Pesticide Program: Residue Monitoring 1992. *J. AOAC Int.* **1993**, *76*, 1193−1205.

Garcia-Cazorla, J.; Xirau-Vayreda, M.; Persistence of Dicarboximidic Fungicide Residues in Grapes, Must, and Wine. *Am. J. Enol. Vitic.* **1994**, *45*, 338−340.

Liñán y Vicente, C. *Vademecum de Productos Fitosanitarios y Nutricionales*; Carlos Liñán y Vicente: Madrid, 1999.

Luke, M. A.; Froberg, J. E.; Masumoto, H. T. Extraction and Cleanup of Organochlorine, Organophosphate, Organonitrogen, and Hydrocarbon Pesticides in Produce for Determination by Gas−Liquid Chromatography. *J.−Assoc. Off. Anal. Chem.* **1975**, *58*, 1020−1026.

MAPA (Spanish Ministry of Agriculture Fisheries and Food). *Informe de los Resultados del Programa Nacional de Vigilancia de Residuos de Productos Fitosanitarios en Origen 1996*; Ministerio de Agricultura, Pesca y Alimentación: Madrid, 1998.

Sala, C.; Fort, F.; Busto, O.; Zamora, F.; Arola, L.; Guasch, J. Fate of Some Common Pesticides During Vinification Process. *J. Agric. Food Chem.* **1996**, *44*, 3668−3671.

Timme, G.; Frehse, H. Statistical Interpretation of the Degradational Behaviour of Pesticide Residues, I. *Pflanzenschutz-Nachr. Bayer*, **1980**, *33*, 47−60.

Tomlin, C., Ed. *The Pesticide Manual*, 10th ed.; British Crop Protection Council: Surrey, UK.; The Royal Society of Chemistry: Cambridge, UK, 1994.

Valverde, A.; González, E. *Análisis de Residuos de Plaguicidas en Hortalizas. Métodos de Extracción*; IEA: Almería, Spain, 1989.

Received for review July 14, 1999. Revised manuscript received April 11, 2000. Accepted April 26, 2000.

JF990770Y