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Iprodione Residues and Dissipation Rates in Tobacco Leaves and Soil

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Abstract Field experiments were conducted in two different locations to determine the residue levels and dissipation rates of iprodione in tobacco leaves and soil. Iprodione 50 % wettable powder formulation was sprayed once at 12.50 g/ha to study the dissipation behavior and three to four times at 8.33 g/ha (recommended dose) and 12.50 g/ha (1.5 times the recommended field dose) to determine the residue levels of iprodione in tobacco leaves and soil after repeated applications. Iprodione residues in both green tobacco leaves and soil dissipated to about 50 % of the initial deposits after 7 days and then further dissipated to more than 90 % after 35 days.The dissipation of iprodione followed first order kinetics and the calculated half-life values ($T_{1/2}$) were 5.64–8.80 days in green tobacco leaves and 7.50–9.93 days in soil, respectively. Iprodione residue levels in flue-cured tobacco leaves 21 days after the third and fourth applications ranged from 7.61 to 40.98 mg/kg. Meanwhile, the residues detected in soil decreased to 0.010–0.117 mg/kg 21 days after the last treatment.

Keywords Iprodione · Residue · Dissipation rate · Tobacco · Soil

Tobacco is an economically important crop consumed not only in China but all over the world. This crop is prone to various insects and diseases at all stages of its development. Among tobacco diseases, brown spot is the second most serious disease in China. It is widely distributed in tobacco-producing regions and causes significant tobacco yield reduction. Iprodione, a dicarboximide fungicide that inhibits DNA and RNA synthesis, cell division and cellular metabolism in fungi (Davidse 1986) is used to control *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Alternaria blight* and other fungal diseases in a variety of greenhouse and field crops such as fruit, vegetables, ornamental trees and scrubs (Adaskaveg and Organia 1994; Smith et al. 1995; Mukherjee et al. 2003). Iprodione has also been proved highly effective against tobacco brown spot and widely used in tobacco fields in China (Shi et al. 2010). The presence of iprodione residues on crops is a matter of serious concern. Although analysis methods for the determination of iprodione residues in different matrics (Rouberty and Fournier 1995; Columé et al. 2000; Avramides et al. 2003) and its behavior in some vegetables and fruit crops such as tomato, apple and strawberry (Omirou et al. 2009; Angioni et al. 2012) have been reported, little information is available about the persistence of iprodione residues in tobacco. The present study was therefore carried out to evaluate iprodione dissipation kinetics and ultimate residues in tobacco leaves and soil under two different field conditons, and thereby provide an evaluation for scientific and safe use of iprodione in tobacco.

Materials and Methods

An analytical standard of iprodione with a purity >97.5 % was purchased from Dr. Ehrenstorfer (Augsburg, Germany) and its formulation 50 % wettable powder (WP) was supplied by Jiangsu Kuaida Agrochemical Co., Ltd. All solvents used, i.e. acetonitrile, acetone, n-hexane, and anhydrous sodium sulfate, were of analytical grade (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China). SPE

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columns (Florisil, 500 mg, 3 mL) were from Agela Technologies (Beijing, China). Stock standard solution of iprodione was prepared with acetone for GC analysis and suitably diluted to obtain the working standards.

Field trials including the dissipation experiments and ultimate residue experiments were carried out at two different locations in China viz., (1) Qingdao, Shandong province and (2) Changsha, Hunan province during 2010–2011. Each treatment, including the untreated control, was replicated thrice in separate plots each of 30 m^2 area. Tobacco plants were planted with a spacing of 1.1 × 0.5 m (1.8 × 10^4 plants/ha). To study the dissipation pattern of iprodione in tobacco and soil, 50 % WP formulation was sprayed at 12.50 g/ha (1.5-folds of recommended dose) with a knapsack sprayer. Green tobacco leaves and soil samples (0–10 cm) were collected randomly from each treatment plot at 0 (2 h after spraying), 1, 3, 5, 7, 14, 21, 28, 35 and 42 days after application. The ultimate residue experiments were performed during tobacco maturing stage. Iprodione 50 % WP formulation was sprayed three and four times every 7 days at 8.33 g/ha (recommended) and 12.50 g/ha (1.5-folds of recommended dose), respectively. About 200 g tobacco leaves and 1 kg soil (0–15 cm) samples were then collected 7, 14 and 21 days after the last iprodione application. The tobacco leaves for the dissipation study were chopped and these for ultimate residue analysis were cured and crushed. Plants roots and large stones were removed from soil samples and then sieved through a 2-mm sieve. Samples were extracted and determined as early as possible.

Green tobacco leaves (20 g) were extracted with 80 mL of acetonitrile using a mechanical shaker at 150 rpm for 1 h, followed by addition of 10 g of sodium chlorideand then continuously shaken for a further 15 min to achieve complete liquid–liquid partition. The extract was dried over anhydrous sodium sulfate and exactly 40 mL of the solvent extract was concentrated to near dryness in a rotary vacuum evaporator at 50°C. After cooling, the extract was reduced just to dryness under weak nitrogen stream, and dissolved again in 2 mL of acetone/n-hexane (1:9, v/v) for further clean-up. A Florisil SPE column was topped with 1 g of anhydrous sodium sulfate and pretreated with 5 mL of acetone/n-hexane (1:9, v/v). The 2-mL extract was transferred to the preconditioned column, eluted from the column with 15 mL of acetone/n-hexane (1:9, v/v) and collected. The eluate was evaporated to dryness, reconstituted in 2 mL of acetone and then filtered through a 0.22 μm filter before GC analysis. For the extraction of iprodione from flue-cured leaves and soil samples, 20 g of soil sampes or 4 g of flue-cured leaves were soaked with an appropriate amount of water for 15 min and then shaken for 1 h in a mechanical shaker. Other extraction steps of iprodione for flue-cured leaves and soil samples were the same as those for green tobacco leaves.

Iprodione compound was identified and quantified using an Agilent 7890A gas chromatography (GC) equipped with a ^{63}Ni electron capture detector (ECD). A capillary column DB-1 (30 m × 0.32 mm i.d. with 0.25 μm film thickness) was used for separation. Oven temperature was initially maintained at 150°C for 2 min, increased to 280°C at a rate of 5°C/min and held at 280°C for 10 min. Injector and detector temperature were held, respectively, at 230 and 300°C. Nitrogen was used as the carrier at a flow rate of 2.0 mL/min. Iprodione concentration was determined using an external calibration curve, with concentrations ranging from 0.01 to 10.0 mg/L. The efficiency and reliability of the method was evaluated by spiking tobacco and soil samples with iprodione working solutions at three fortification levels (0.01, 1.0, 10.0 mg/kg). The amount of recovered iprodione was analyzed following the above analytical method.

Results and Discussion

A good linearity was achieved in the range of 0.01–10.0 mg/L with a correlation coefficient of 0.9998. Mean recoveries and standard deviations (SD) are shown in Table 1. The mean recoveries of iprodione in green and flue-cured tobacco leaves ranged from 85.54 %–103.40 % to 87.03 %–94.57 %, respectively and SD of 3.46 %–6.78 %. For soil, the mean recoveries were in the range of 93.26 %–94.57 % with SD values in the range 3.21 %–5.89 %. The limit of detection (LOD) and limit of quantification (LOQ) were established at the signal-to-noise ratio (S/N) of 3:1 and 10:1, respectively. The LOD and LOQ in this study were determined as 0.003 and 0.01 mg/kg, respectively, for both tobacco and soil samples.

Data on iprodione dissipation in green tobacco leaves and soil are presented in Tables 2 and 3, respectively. No iprodione residue was detectable in any analyzed control

Table 1 Recovery of iprodione in tobacco and soil samples at different fortification levels

Substrate	Fortification level (mg/kg)	Recovery % (mean ± SD, n = 5)
Green tobacco leaf	0.01	103.40 ± 4.73
	1.0	92.54 ± 3.46
	10.0	85.54 ± 5.19
Flue-cured tobacco leaf	0.01	87.03 ± 6.33
	1.0	94.57 ± 4.52
	10.0	93.73 ± 6.78
Soil	0.01	94.18 ± 5.43
	1.0	93.26 ± 3.21
	10.0	94.57 ± 5.89

Table 2 Dissipation of iprodione in green tobacco leaves

Days after application	Residue[a] in mg/kg (% of dissipation)			
	Qingdao		Changsha	
	2010	2011	2010	2011
0	29.40 (–)	23.66 (–)	37.19 (–)	39.84 (–)
1	27.31 (7.11)	22.36 (5.49)	32.32 (13.09)	34.49 (13.43)
3	22.92 (22.04)	16.23 (31.40)	28.84 (22.45)	26.64 (33.13)
5	12.56 (57.28)	13.76 (41.84)	20.32 (45.36)	21.73 (45.46)
7	10.28 (65.03)	12.14 (48.69)	15.97 (57.06)	19.32 (51.51)
14	8.51 (71.05)	10.49 (55.66)	8.42 (77.36)	13.45 (66.24)
21	5.43 (81.53)	8.26 (65.09)	2.36 (93.65)	7.92 (80.12)
28	2.64 (91.02)	5.20 (78.02)	2.29 (93.84)	4.71 (88.18)
35	0.51 (98.27)	1.23 (94.80)	2.09 (94.38)	2.03 (94.90)
42	0.07 (99.76)	0.56 (97.63)	0.33 (99.11)	0.27 (99.32)

[a] Average of three replicates

Table 3 Dissipation of iprodione in soil

Days after application	Residue[a] in mg/kg (% of dissipation)			
	Qingdao		Changsha	
	2010	2011	2010	2011
0	0.390 (–)	0.343 (–)	0.132 (–)	0.253 (–)
1	0.323 (17.18)	0.299 (12.83)	0.118 (16.57)	0.191 (24.51)
3	0.222 (43.08)	0.288 (16.03)	0.101 (31.49)	0.165 (34.78)
5	0.212 (45.64)	0.182 (46.94)	0.091 (45.86)	0.141 (44.27)
7	0.207 (46.92)	0.141 (58.89)	0.069 (55.25)	0.112 (55.73)
14	0.153 (60.77)	0.055 (83.97)	0.031 (63.54)	0.102 (59.68)
21	0.103 (73.59)	0.036 (89.50)	0.024 (68.51)	0.063 (75.10)
28	0.057 (85.38)	0.014 (95.92)	ND (-)	0.033 (86.96)
35	0.027 (93.08)	0.011 (96.79)	ND (-)	0.017 (93.28)
42	0.010 (97.44)	0.010 (97.08)	ND (-)	0.011 (95.65)

[a] Average of three replicates: ND: <LOD

tobacco and soil samples. The initial deposits of iprodione in green tobacco leaves were in the range of 23.66–39.84 mg/kg. The differences in the initial deposits were probably due to the different sizes of tobacco leaves. Treatments carried out with equal volumes of iprodione would give different residue levels and tobacco leaves with the lower surface/weight ratio trapped lower residues (Angioni et al. 2012). Seven days after application, iprodione residues dissipated to about 50 % of the initial deposits and then further dissipated to more than 90 % after 35 days irrespective of any experimental locations and years. The dissipation of iprodione in green tobacco leaves followed the first-order degradation kinetics with the half-life values ($T_{1/2}$) varying from 5.64 to 8.80 days (Table 4). The initial concentrations of iprodione in soil were in the range of 0.132–0.390 mg/kg. The residues

Table 4 Dissipation kinetics of iprodione in green tobacco leaves and soil

Site	Years	Substrate	Regression equation	R^2	$T_{1/2}$ (days)
Qingdao	2010	Tobacco	$C = 34.157e^{-0.1228t}$	0.9172	5.64
		Soil	$C = 0.3644e^{-0.0759t}$	0.9599	9.13
	2011	Tobacco	$C = 25.052e^{-0.0788t}$	0.9158	8.80
		Soil	$C = 0.2903e^{-0.0924t}$	0.9595	7.50
Changsha	2010	Tobacco	$C = 34.794e^{-0.1013t}$	0.9524	6.84
		Soil	$C = 0.1295e^{-0.0866t}$	0.9730	8.00
	2011	Tobacco	$C = 42.781e^{-0.0987t}$	0.9254	7.02
		Soil	$C = 0.2202e^{-0.0698t}$	0.9817	9.93

dissipated above 50 % of the initial deposits 7 days after treatment except that in Qingdao in the year 2010 which remained 53.08 % of the initial deposit. The residues in soil dissipated more than 90 % after 35 days. Iprodione seemed to dissipate slower in soil than in green tobacco leaves. The half-life values were observed to be 7.50–9.93 days in soil (Table 4). Dissipation rates of iprodione might be affected by several factors such as pesticide properties, soil characteristics, environmental conditions, etc. One more important factor to consider could be growth dilution. With the size and weight of tobacco leaves increasing, residues were observed to dissipate rapidly. These data are consistent with earlier studies which showed half-lives of 6.02 days in mustard leaves (Mukherjee et al. 2003), 10.50 days in tomato fruits (Omirou et al. 2009), and 9.52–10.95 days in soil (Song et al. 2004), respectively.

Mature tobacco leaves were harvested 7, 14 and 21 days after the third and fourth applications. As the maximum residue limits (MRLs) for pesticides in tobacco have been established according to residue levels in cured tobacco leaves, the harvested leaves were cured by conventional processes (Liu and Liu 2010) and then the residue levels of iprodione in flue-cured leaves and soil samples were determined. The results of ultimate residues in flue-cured tobacco leaves and soil are shown in Tables 5 and 6, respectively. The iprodione residues in cured tobacco leaves 7 days after the last application ranged from 26.48 to 79.77 mg/kg, and after 21 days dissipated rapidly to 7.61–40.98 mg/kg. Meanwhile, the residues detected in

Table 5 Ultimate iprodione residues in flue-cured tobacco leaves at different time intervals after application

Dosage (g/ha)	Spray times	Interval (days)	Average residue[a] (mg/kg)			
			Qingdao		Changsha	
			2010	2011	2010	2011
8.33	3	7	38.88	26.48	28.88	38.01
		14	20.00	14.86	19.28	22.59
		21	13.50	12.02	10.45	7.61
	4	7	44.74	30.76	26.55	66.61
		14	33.61	21.53	13.46	44.99
		21	23.03	11.45	11.96	22.57
12.50	3	7	53.18	40.89	39.88	68.79
		14	42.59	34.89	21.73	48.58
		21	34.17	22.88	22.14	29.58
	4	7	60.24	55.11	45.31	79.77
		14	45.94	33.67	39.14	73.14
		21	36.97	28.54	32.47	40.98

[a] Each value is the mean of three replicates

Table 6 Ultimate iprodione residues in soil at different time intervals after application

Dosage (g/ha)	Spray times	Interval (days)	Average residue[a] (mg/kg)			
			Qingdao		Changsha	
			2010	2011	2010	2011
8.33	3	7	0.091	0.058	0.160	0.168
		14	0.053	0.037	0.118	0.104
		21	0.046	0.052	0.080	0.010
	4	7	0.163	0.045	0.231	0.212
		14	0.082	0.027	0.139	0.121
		21	0.053	0.012	0.104	0.091
12.50	3	7	0.130	0.049	0.108	0.395
		14	0.067	0.035	0.228	0.218
		21	0.038	0.027	0.117	0.090
	4	7	0.116	0.047	0.254	0.264
		14	0.054	0.036	0.221	0.098
		21	0.041	0.022	0.098	0.025

[a] Each value is the mean of three replicates

soil reached 0.010–0.117 mg/kg 21 days after the last treatment. During the curing process, tobacco leaves are dried and decreased in weight by about 85 %. Although iprodione residues were lost to some degree after high-temperature curing, residues in cured tobacco leaves showed much higher levels than that in mature leaves which were not cured. MRL for iprodione in tobacco has not been established and the risk assessment of iprodione residues in tobacco can be carried out according to a MRL of 25 mg/kg in lettuce leaf established by FAO/WHO. Our results show that iprodione (50 % WP) can be applied in tobacco no more than four times at a dosage not exceeding 8.33 g/ha. There should be a withholding period of at least 21 days between the last application and harvest. Although cured tobacco leaves will be aged for more than 1 year before they are processed into cigarettes and iprodione residues will decrease during the process, whether iprodione can be applied in tobacco at higher doses should be evaluated further.

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References

Adaskaveg JE, Organia JM (1994) Penetration of iprodione into mesccarp fruit tissue and suppression of gray mold and brown rot of sweet cherries. Plant Dis 78:293–296

Angioni A, Porcu L, Dedola F (2012) Determination of famoxadone, fenamidone, fenhexamid and iprodione residues in greenhouse tomatoes. Pest Manag Sci 68(4):543–547

Avramides EJ, Lentza-Rizos C, Mojasevic M (2003) Determination of pesticide residues in wine using gas chromatography with nitrogen-phosphorus and electron capture detection. Food Addit Contam 20(8):699–706

Columé A, Cárdenas S, Gallego M, Valcárcel M (2000) Simplified method for the determination of chlorinated fungicides and insecticides in fruits by gas chromatography. J Chromatogr A 882:193–203

Davidse LC (1986) Benzimidazole fungicides: mechanism of action and biological impact. Annu Rev Phytopathol 24:43–65

Liu GS, Liu JL (2010) Practical guide to leaf tobacco production techniques in China. China National Leaf Tobacco Corporation, Beijing, pp 194–200

Mukherjee I, Gopal M, Chatterjee SC (2003) Persistence and effectiveness of iprodione against *Alternaria blight* in mustard. Bull Environ Contam Toxicol 70:586–591

Omirou M, Vryzas Z, Papadopoulou-Mourkidou E, Economou A (2009) Dissipation rates of iprodione and thiacloprid during tomato production in greenhouse. Food Chem 116(2):499–504

Rouberty F, Fournier J (1995) Capillary gas chromatographic-Mass spectrometric determination of iprodione in chicory and leek. Chromatographia 41:693–696

Shi YP, Chen J, Jia ZJ (2010) Bioassay and field trials of iprodione on *Alternaria alternate*. Mod Agrochem 9:54–56

Smith FD, Phipps DM, Stipes RJ, Brenneman TB (1995) Significance of insensitivity of Sclerotinia minor to iprodione in control of *Sclerotinia blight* of peanut. Plant Dis 79:517–523

Song GC, Yu JL, Zhang JQ, Lei M, Zhao DY, Pan HL (2004) The dynamics of iprodione residue disappearance in apples and soil. Agrochem 43(4):184–185