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Dissipation and Residues of Myclobutanil in Tobacco and Soil Under Field Conditions

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Abstract Field experiments were conducted in two different locations to determine the dissipation pattern and residue levels of myclobutanil in tobacco leaves and soil. Myclobutanil 12.5 % microemulsion (ME) formulation was sprayed once at 3.0 mL/ha, and the residues in green tobacco leaves dissipated to more than 50 % of the initial deposits 5 days after application and up to above 90 % after 21 days. The dissipation rate of myclobutanil in soil was lower than that in green tobacco leaves. The residues dissipated above 50 % of the initial deposits 7 days after treatment and dissipated about 90 % after 42 days. The calculated half-life values ($T_{1/2}$) were found to be 4.89–6.77 days in green tobacco leaves and 12.88–19.20 days in soil, respectively. The ultimate residues of myclobutanil in flue-cured tobacco leaves and soil were determined after the third and fourth applications at levels of 2.0 and 3.0 mL/ha. Myclobutanil residues in cured tobacco leaves 21 days after the last treatment ranged from 0.85 to 3.27 mg/kg. Meanwhile, the residues detected in soil reached below 0.045 mg/kg 21 days after the last treatment.

Keywords Myclobutanil · Tobacco · Residue · Dissipation

Myclobutanil is a systemic fungicide belonging to the triazole family of chemicals. The basis of its fungicidal action is the inhibition of sterol 14-demethylase enzyme, which produces ergosterol, an organic compound vital to fungal cell wall formation (Hassali 1990). Myclobutanil has therefore been registered as an agricultural fungicide (Székács and Hammock 1995) and been used on a wide range of crops including cereals, vegetables, pome and stone fruits. Myclobutanil is easily absorbed by the foliage and moved efficiently in the xylem and apoplast, showing strong curative activity and efficacy (Koller and Wubben 1989). Nevertheless, myclobutanil displays no preventive activity and emerging fungal resistance against it has been reported (Braun and McRae 1992), which require more frequent and increased volume applications. Tobacco powdery mildew (*Erysiphe cichoracearum* DC.) and tobacco brown spot (*Alternaria alternata* (Fries) Keissler) are serious diseases for tobacco production in China. Although myclobutanil is high effective against these two tobacco diseases and used widely in tobacco field, the abuse of myclobutanil is a matter of environmental concern. Myclobutanil has been found even in surface water and rain and may accumulate with multiple applications due to its persistence (Vogel et al. 2008). Previous work have been reported about myclobutanil residues on vegetables and fruits (Athanasopoulos et al. 2003; Lou et al. 2008), little information is available for the assessment of myclobutanil residue dissipation in tobacco in China.

The purpose of the present work is to study dissipation pattern as well as ultimate residues of myclobutanil in tobacco and soil under field conditions, and thereby provide an evaluation for scientific, safe use of myclobutanil.

Materials and Methods

An analytical standard of myclobutanil with a purity >97 % was purchased from Dr. Ehrenstorfer (Augsburg,

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Germany) and its formulation 12.5 % microemulsion (ME) was supplied by Beijing Dongwang (China) 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). Stock standard solution of myclobutanil 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 in two different locations in China viz., (1) Qingdao, Shandong province and (2) Changsha, Hunan province during 2009–2010. For each treatment, three replicate plots of 30 m^2 each and a control plot without myclobutanil application were used and distributed in a randomized block design. Tobacco plants were planted with a spacing of 1.1 × 0.5 m (1.8 × 10^4 plants/ha). To study the dissipation pattern, myclobutanil 12.5 % ME formulation was sprayed at 3.0 mL/ha (1.5 times recommended field dose) with a knapsack sprayer. Fresh 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 myclobutanil application. The ultimate residue experiments were performed during tobacco maturing stage. Myclobutanil 12.5 % ME formulation was sprayed three and four times every 7 days at concentrations of 2.0 and 3.0 mL/ha, corresponding to recommended field dose and 1.5 times the recommended field 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 application of myclobutanil. 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 as early as possible. If not possible, they were stored at −20°C for further myclobutanil determination.

For the extraction of myclobutanil from tobacco samples, 10 g of green leaves or 4 g of flue-cured leaves were transferred into a 100-mL Erlenmeyer flask, followed by addition of 80 mL of 1 % acetic acid/acetonitrile. The tobacco solvent mixture was shaken for 1 h at 150 rpm on a mechanical shaker, followed by addition of sodium chloride (10–15 g) and then continuously shaken for a further 15 min to complete liquid–liquid partition. The extract was dried over anhydrous sodium sulfate and exactly 40 mL of the same extracting solvent was concentrated to near dryness in a rotary vacuum evaporator at 40°C and then dried with weak nitrogen stream without disturbing the surface of the extract. The residue was dissolved again in 5 mL of acetone/n-hexane (20:80, v/v) for further clean-up. Sodium sulphate (1 cm) was added to a Florisil SPE cartridge and the column was pretreated with 5 mL of acetone/n-hexane (20:80, v/v) without drying. The concentrated acetone/n-hexane extract was transferred to the preconditioned column, eluted from the column with 15 mL of acetone/n-hexane (20:80, v/v) and collected. The eluate was evaporated to dryness, reconstituted in 2 mL of n-hexane and then filtered through a 0.45 μm filter before GC analysis. Soil samples (20 g) 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 myclobutanil from soil samples were the same as those from tobacco samples without SPE clean-up.

Myclobutanil residue was determined by an Agilent 6890 N gas chromatography (GC) equipped with an electron capture detector (ECD) ^{63}Ni. A capillary column DB-1 (30 m × 0.32 mm i.d. × 0.25 μm film thickness) was used for separation. The injector and detector temperature were maintained at 230 and 300°C, respectively. The column temperature was initially maintained at 150°C for 1 min, increased at 5°C/min to 280°C and held at the final temperature for 10 min. Nitrogen was used as the carrier and make-up gas with a constant flow rate of 30 mL/min. Hydrogen and air were used as detector gases at 4 and 175 mL/min, respectively. The injection volume was 1 μL in the splitless mode. Under the experimental conditions described, the GC retention time for myclobutanil was about 14.3 min. Recovery studies were carried out to assess the efficiency and reliability of the method. The untreated samples (tobacco leaves and soils) were fortified with analytical grade myclobutanil at rates of 0.01, 0.1 and 1.0 mg/kg, respectively. The amount of recovered myclobutanil was analyzed following the above analytical method.

Results and Discussion

Standard calibration curve was constructed from the peak area of the calibration run. Good linearity was achieved in the range of 0.01–10.0 mg/kg. The concentration of residual myclobutanil in the extract was determined by comparing the peak area with that of the reference standard. The recovery study was carried out five replicates at three spiked levels (0.01, 0.1 and 1.0 mg/kg). The average recoveries obtained ranged from 88.53 % to 104.16 % in green tobacco leaf samples, 91.46 %–100.66 % in flue-cured leaf samples, and 86.34 % to 105.26 % in soil samples, respectively (Table 1). The limit of detection (LOD) and limit of quantification (LOQ) were established when the signal-to-noise ratios (S/N) were 3:1 and 10:1, respectively. The LOD and LOQ in this study were determined as 0.01 and 0.03 mg/kg, respectively, for both tobacco and soil samples.

Table 1 Average recovery of myclobutanil in samples spiked at different levels

Substrate	Spiked level (mg/kg)	Recovery % (Mean ± SD, n = 5)
Green tobacco leaf	0.01	104.16 ± 4.07
	0.1	95.84 ± 3.47
	1.0	88.53 ± 6.61
Flue-cured tobacco leaf	0.01	91.46 ± 5.42
	0.1	95.34 ± 4.44
	1.0	100.66 ± 2.04
Soil	0.01	105.26 ± 5.10
	0.1	94.57 ± 4.50
	1.0	86.34 ± 2.75

The dissipation pattern of myclobutanil in green tobacco leaves during 2009–2010 is shown in Table 2. The initial deposits (2 h after spraying) of myclobutanil in Qingdao were found to be 9.81 mg/kg in 2009 and 7.83 mg/kg in 2010, respectively. The initial concentrations of myclobutanil in Changsha were 8.94 mg/kg in 2009 and 6.84 mg/kg in 2010, respectively. No residues were detected in the control. As larger leaf surface traps higher amounts of pesticides than the smaller one, the differences in initial myclobutanil concentration at day 0 were probably due to the different sizes of tobacco leaves which grew at different environmental conditions in various tobacco growing regions. The residues of myclobutanil in green tobacco leaves declined progressively with time. It was found that the dissipation was faster initially and slowed down with the passage of time. Five days after application, myclobutanil residues dissipated to more than 50 % of the initial deposits irrespective of any experimental locations and years, which further dissipated to above 90 % after 21 days. The dissipation of myclobutanil in green tobacco leaves followed the first-order kinetics with the half-life values ($T_{1/2}$) varying from 4.89 to 6.77 days (Table 4). The dissipation data of myclobutanil in soil is presented in

Table 2 Dissipation of myclobutanil in green tobacco leaves

Days after application	Residue[a] in mg/kg (% of dissipation)			
	Qingdao		Changsha	
	2009	2010	2009	2010
0	9.81 (–)	7.83 (–)	8.94 (–)	6.84 (–)
1	8.48 (13.56)	6.76 (13.67)	6.96 (22.15)	5.99 (12.43)
3	7.30 (25.59)	6.43 (17.88)	5.60 (37.36)	5.01 (26.75)
5	4.89 (50.15)	3.59 (54.15)	3.66 (59.06)	2.82 (58.77)
7	3.01 (69.32)	1.30 (83.40)	1.61 (81.99)	1.50 (78.07)
14	1.77 (81.96)	0.42 (94.64)	1.19 (86.69)	0.59 (91.37)
21	0.85 (91.34)	0.22 (97.19)	0.41 (95.41)	0.24 (96.49)
28	0.73 (92.56)	0.20 (97.45)	0.20 (97.76)	0.16 (97.66)
35	0.35 (96.43)	0.11 (98.60)	0.17 (98.10)	0.03 (99.56)
42	0.08 (99.18)	0.06 (99.23)	0.09 (98.99)	0.02 (99.71)

[a] Average of three replicates

Table 3 Dissipation of myclobutanil in soil

Days after application	Residue[a] in mg/kg (% of dissipation)			
	Qingdao		Changsha	
	2009	2010	2009	2010
0	0.194 (–)	0.196 (–)	0.181 (–)	0.248 (–)
1	0.162 (16.49)	0.137 (30.10)	0.151 (16.57)	0.206 (16.94)
3	0.143 (26.29)	0.111 (43.37)	0.124 (31.49)	0.182 (26.61)
5	0.131 (32.47)	0.107 (45.41)	0.098 (45.86)	0.136 (45.16)
7	0.091 (53.09)	0.095 (51.53)	0.081 (55.25)	0.116 (53.23)
14	0.071 (63.40)	0.085 (56.63)	0.066 (63.54)	0.077 (68.95)
21	0.064 (67.01)	0.071 (63.78)	0.057 (68.51)	0.054 (78.23)
28	0.052 (73.20)	0.063 (67.86)	0.044 (75.69)	0.044 (82.26)
35	0.042 (78.35)	0.042 (78.57)	0.031 (82.87)	0.030 (87.90)
42	0.028 (85.57)	0.028 (85.71)	0.017 (90.61)	0.024 (90.32)

[a] Average of three replicates

Table 3. The initial concentrations of myclobutanil in soil were in the range of 0.181–0.248 mg/kg. Dissipation rates of myclobutanil in soil were lower than that in green tobacco leaves. The residues dissipated above 50 % of the initial deposits 7 days after treatment and dissipated about 90 % after 42 days. The dissipation pattern of myclobutanil in soil could also be explained in terms of first order reaction and the calculated half-life values were found to be 12.88–19.20 days (Table 4). The results were in accordance with the previous studies which showed that 45.83 % of the initial concentrations in strawberry fruits dissipated in the first 4 days after application and the half-lives of myclobutanil were 5.17 days in strawberry (Sobeiha et al. 2009), 3.4 days in onion (Cui et al. 2011), and 14.1–14.3 days in soil (Lou et al. 2008), respectively.

Mature tobacco leaves were harvested 7, 14 and 21 days after the last application. As the maximum residue limits (MRLs) for pesticides in tobacco have been established normally according to residue present in cured tobacco leaves, the harvested leaves were cured by conventional processes (Liu and Liu 2010) and then the residue levels of myclobutanil in flue-cured leaf and soil samples were determined. The ultimate residue results were shown in Tables 5 and 6. The myclobutanil residues in cured tobacco leaves 7 days after the last application ranged from 6.49 to 25.19 mg/kg, and after 21 days dissipated rapidly to 0.85–3.27 mg/kg. Meanwhile, the residues detected in soil reached below 0.045 mg/kg 21 days after the last treatment. As the cured tobacco leaves are not suitable for cigarette products because of its disagreeable odor and undesirable aroma, the aging process of tobacco is necessary after flue-curing, which is a key step to improve tobacco aroma quality and to increase its usability (Huang et al. 2010). Normally, the cured tobacco leaves need to be aged for 2–3 years before they are manipulated again. During the long-term aging process, myclobutanil residues in cured tobacco leaves will decrease further. In addition, high temperature pyrolysis of myclobutanil may occur during the smoking. Thus, the dissipation pattern of myclobutanil at various stages of aging and smoking processes should be evaluated further.

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

Site	Year	Substrate	Regression equation	R^2	$T_{1/2}$ (days)
Qingdao	2009	Tobacco	$C = 8.666e^{-0.1024t}$	0.9705	6.77
		Soil	$C = 0.1566e^{-0.0409t}$	0.9494	16.95
	2010	Tobacco	$C = 5.352e^{-0.118t}$	0.919	5.87
		Soil	$C = 0.1439e^{-0.0361t}$	0.9269	19.20
Changsha	2009	Tobacco	$C = 6.152e^{-0.1093t}$	0.9575	6.34
		Soil	$C = 0.1444e^{-0.0476t}$	0.9553	14.56
	2010	Tobacco	$C = 5.851e^{-0.1417t}$	0.983	4.89
		Soil	$C = 0.1966e^{-0.0538t}$	0.9692	12.88

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

Dosage (mL/ha)	Spray times	Interval (days)	Average residue[a] (mg/kg)			
			Qingdao		Changsha	
			2009	2010	2009	2010
3.0	3	7	15.26	16.37	10.86	11.03
		14	8.66	8.20	3.14	5.58
		21	2.86	2.04	2.62	1.15
	4	7	22.61	16.35	16.04	25.19
		14	10.07	8.67	4.35	10.79
		21	3.27	2.39	2.60	2.06
2.0	3	7	11.82	12.52	6.49	9.92
		14	6.04	7.27	2.06	2.88
		21	2.51	2.12	1.83	0.85
	4	7	11.84	12.28	8.40	11.64
		14	7.01	7.60	2.06	6.40
		21	2.73	2.68	1.97	1.31

[a] Each value is the mean of three replicates

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

Dosage (mL/ha)	Spray times	Interval (days)	Average residue[a] (mg/kg)			
			Qingdao		Changsha	
			2009	2010	2009	2010
3.0	3	7	0.010	0.082	ND	0.061
		14	0.013	0.050	ND	0.047
		21	ND	0.023	ND	ND
	4	7	0.025	0.081	0.016	0.110
		14	0.022	0.062	0.015	0.081
		21	0.016	0.044	ND	0.026
2.0	3	7	0.011	0.053	ND	0.073
		14	ND	0.042	ND	0.052
		21	ND	0.029	ND	0.037
	4	7	0.014	0.113	0.014	0.074
		14	0.011	0.081	0.013	0.054
		21	0.013	0.045	ND	0.030

[a] Each value is the mean of three replicates; ND: < LOD

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References

Athanasopoulos PE, Pappas CJ, Kyriakidis NV (2003) Decomposition of myclobutanil and triadimefon in grapes on the vines and during refrigerated storage. Food Chem 82:367–371

Braun PG, McRae KB (1992) Composition of a population of *Ventura inaequalis* resistant to myclobutanil. Can J Plant Pathol 14:215–220

Cui SH, Wang KY, Yang J, Qian JL, Liu JF, Zhang LD (2011) Residue dynamics of myclobutanil in onion. Agrochemicals 50:283–288

Hassali KA (1990) The biochemistry and uses of pesticides. MacMillan, London

Huang JW, Yang JK, Duan YQ, Gu W, Gong XW, Zhe W, Su C, Zhang KQ (2010) Bacterial diversities on unaged and aging flue-cured tobacco leaves estimated by 16S rRNA sequence analysis. Appl Microbiol Biotechnol 88:553–562

Koller W, Wubben JP (1989) Variable resistance factors of fungicides acting as sterol demethylation inhibitors. Pestic Sci 26:133–145

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

Lou ZY, Tang FB, Luo FJ, Liu GM, Chen ZM (2008) Residue dynamics of myclobutanil in cucumber and soil. Agrochemicals 47:205–207

Sobeiha AK, Abdel-Megeed MI, Dahroug SMA, Eklo OM, Hanafi A (2009) Disappearance of azoxystrobin, cyprodinil, fludioxonil, fenhexamid and myclobutanil in strawberry fruits under field conditions. Arab Univ J Agric Sci 17:239–245

Székács A, Hammock BD (1995) Development of an enzyme-linked immunosorbent assay for the detection of the triazole fungicide myclobutanil. J Agric Food Chem 43:2083–2091

Vogel JR, Majewski MS, Capel PD (2008) Pesticides in rain in four agricultural watersheds in the United States. J Environ Qual 37:1101–1115