The dissipation rates of myclobutanil and residue analysis in wheat and soil using gas chromatography-ion trap mass spectrometry

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The dissipation of myclobutanil (triazole fungicide) in wheat plant, grain and soil under the local weather and soil conditions was studied and fungicide residues were determined by GC-ITMS. Myclobutanil (25% EC) was applied at two dosages, 60 g a.i. ha^{-1} (recommended) and 120 g a.i. ha^{-1} (2 times of the recommended dosage) in the experimental fields in Shandong, Beijing and Henan provinces (experimental localities). The detection limit (LOD) and the limit of quantification (LOQ) of myclobutanil were 0.6 µg kg^{-1} and 2 µg kg^{-1} respectively, and they were much below the maximum residue limit (MRL 0.02 mg kg^{-1}) in the EU. The average recoveries were 76.4–97.7, 82.6–92.6 and 75.0–94.4% with relative standard deviation (RSD) of 6.3–15.9, 3.8–17.3 and 3.0–19.7% in wheat plant at four spiking levels (0.01, 0.1, 0.5, 10 mg kg^{-1}), wheat grain at four spiking levels (0.005, 0.01, 0.1, 10 mg kg^{-1}) and soil at five spiking levels (0.002, 0.01, 0.1, 0.5, 10 mg kg^{-1}), respectively. The results showed that the half-lives of myclobutanil in wheat plant and soil from Shandong, Beijing and Henan were 3.5–4.5 and 11.0–11.7 days, respectively. At harvest, wheat grain samples were found to contain myclobutanil well below the MRL following the recommended dosage and the interval of 20 days after last application.

Keywords: fungicide residue; dissipation; gas chromatography-ion trap mass spectrometry; myclobutanil; wheat; soil

1. Introduction

Agricultural chemicals have been widely used all over the world, and they have allowed the development of agricultural and farming production by controlling a wide range of pests and diseases. However, it is well known that the application of these chemicals can be detrimental to human health, as well as the environment [1,2]. Dissipation studies of pesticides are conducted to provide a more realistic result of what happened in the environment. From these data, a quantitative environmental fate profile or assessment and model estimates of exposure to the pesticide in crop, vegetables, fruits or soil under field conditions can be obtained [3–7].

Cereal grains are raw materials for the main foods at the basis of all of the regional diets in the world. In China, wheat (*Triticum aestivum L.*) is the second-most important crop, next to rice [8]. Powdery mildew of wheat causes significant reductions in yield, test

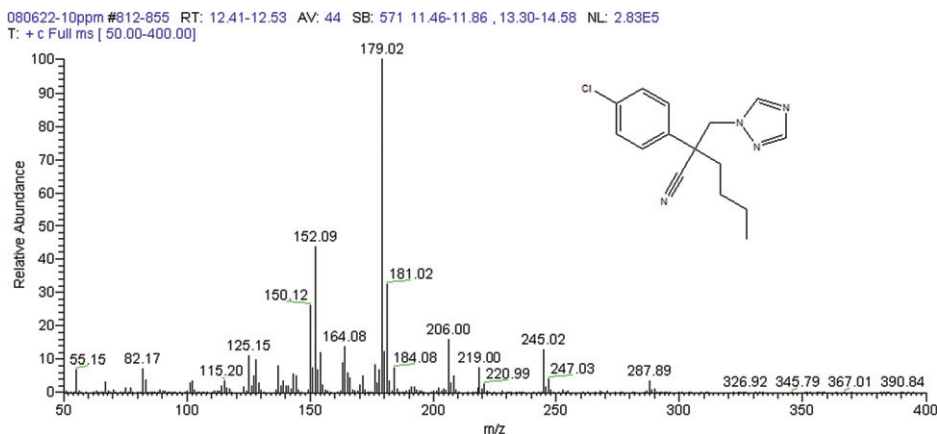*Corresponding author. Email: yqzheng@ippcaas.cn

Figure 1. Mass spectra and structure of myclobutanil.

weight and seed quality, and the chemical control of this disease is always the first choice because it is rapidly effective.

Myclobutanil, chemically known as 2-(4-chlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)hexanenitrile (Figure 1), was a kind of triazole fungicide with the function of protection, curative and systemic trait. As a broad spectrum fungicide, myclobutanil acts by interfering with ergosterol biosynthesis by inhibiting demethylation of steroids [9–11]. It was extremely active against the powdery mildew and rust of grain, and some reports related the use and efficacy of myclobutanil in crops, fruits and vegetables to improve their yield and quality [12–14].

Traditionally, conventional multi-residue methods have been performed for the analysis of myclobutanil residue in the extracts by gas chromatography [15]. However, analytical methodologies must be able to measure residues at very low concentration levels and must also provide unambiguous evidence to confirm both the identity and the magnitude of any residue detected. Gas chromatography/mass spectrometry (GC/MS) has been used to determine pesticide residues in fresh vegetables and wine [16–19]. The use of MS/MS mode improves the selectivity of the technique, sometimes with a drastic reduction in the background and without loss of identification capability. It enables analysis of pesticides at trace levels in the presence of many matrix interferences [20,21]. GC/MS/MS, including the ion trap mass spectrometer (ITMS), is particularly useful for the analysis of complex mixtures, because it enables the separation and identification of components of different structures eluted at similar retention times and at widely different concentrations [22].

In recent years important research work has been carried out to establish the behaviour of myclobutanil in wine distillates [17], and the behaviour of pesticide residue on grapes, from treatment to harvest, and their fate in drying, wine-making, and alcoholic beverage processing [23,24]. In addition, Navarro *et al.* described the fate of triazole fungicide from barley to malt [25].

However, an efficient analytical method for the dissipation rates of myclobutanil and residue analysis in wheat and soil by gas chromatography-ion trap mass spectrometry are still lacking. Therefore, in this work we established the residue analytical methods for myclobutanil in wheat and soil using GC-ITMS with MS/MS mode, and investigated the

persistence, dissipation and kinetics of myclobutanil residues in wheat crops and the soil in which they were grown.

2. Experimental

2.1 *Materials and standards*

The analytical standard of myclobutanil (99.0% purity) and 25% emulsifiable concentrate were obtained from the manufacturer pesticide factory of the Institute of Plant Protection, Chinese Academy of Agricultural Sciences (Beijing, China). The solubility of myclobutanil in water is $142 \, mg \, L^{-1}$ (25°C), and the organic-carbon sorption constant (K_{OC}) is $517 \, mL \, g^{-1}$. Myclobutanil has a boiling point of 202–208°C and vapour pressure at 25°C of 0.198 mPa. The LD_{50} (rat) of myclobutanil is $1600 \, mg \, kg^{-1}$. Methanol, *n*-hexane, acetone, acetonitrile, dichloromethane, NaCl and Na_2SO_4 for pesticide residue analysis were analytical grade purchased from Beihua fine-chemicals Co., Beijing. Purified water was obtained from Wahaha Co., Hangzhou. Gas chromatography grade acetone and *n*-hexane were supplied by Sigma-Aldrich (Steinheim, Germany). Sep-Pak silica cartridge column was Model cleanert C18, 500 mg/6 mL, from Agela Technologies Inc. (Beijing, China).

2.2 *Preparation of standard solutions*

Fungicide individual stock solution standard ($1 \, g \, L^{-1}$) was prepared in *n*-hexane and stored in dark vials at −20°C. The standard stock solution as well as working solutions were prepared in *n*-hexane and were stable for 6 months. Myclobutanil standard solutions (5–$500 \, \mu g \, L^{-1}$) for calibration and quantitation were prepared in *n*-hexane and in wheat plant, wheat grain and soil extracts (matrix-matched standard solutions). Matrix-matched standards solutions were prepared by evaporating to dryness portions of the final extract solution obtained from the matrices (untreated wheat plant, wheat grain and soil without myclobutanil) under gentle nitrogen stream and taking up the remained residue with the calibration solution.

2.3 *Field trials*

The field trials were conducted in three experimental fields located in Shandong, Henan and Beijing during the agricultural season in April 2008. The maximum day temperatures were in the range of 13–33°C during the experimental process in Shandong, Beijing and Henan. There was a light rainfall at 6 days (about 4.2–5.4 mm) and 8 days (about 1.6–2.6 mm) after application in the dissipation study in Shandong. The soils characteristics of three districts were listed in Table 1. The field was divided into $30 \, m^2$-sized blocks for the controls as well as the dissipation rate study. A buffer area separated plots used for different treatments in each field. In addition, the dissipation study of soil was conducted in the bare soil. The wheat plant and bare soil were both, in three replications, sprayed with 25% EC of myclobutanil at a dosage of 120 g active ingredient in 900 L water per hectare (2 times the recommended dosage). Soil samples were collected from different depths ranging from 0 to 15 cm at 15 randomly selected sampling points in each plot. The soil samples were collected at 2 h, 1, 3, 7, 14, 21, 28, 35, 42, 60 and 90 days. The wheat plant (included the leaves and stems) samples were collected at 2 h, 1, 3, 7, 14,

Table 1. Textural and chemical properties of soils used in the study.

	Type	Texture	N (mg kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	pH	CEC[a] (cmol kg^{-1})	Total (%) C	Total (%) N	OM[b] (%)
Shandong	Alluvial	Clay loam	185	58.3	233	7.0	12.9	1.34	0.16	2.7
Beijing	Alluvial	Sandy loam	341	23.3	357	6.7	14.8	0.78	0.11	4.5
Henan	Alluvial	Clay loam	99.0	11.8	171	6.8	14.0	1.18	0.10	1.9

Notes: [a]Cation exchange capacity.
[b]Organic matter content.

21, 28 and 35 days after spraying. Both plant and soil samples were put into polyethylene bags, transported to the laboratory and stored at $-20°C$ until analysed.

To investigate the effects of myclobutanil dosage and spray frequency, a two-by-two experimental design was used. High (120 g a.i. ha.$^{-1}$) and low (60 g a.i. ha.$^{-1}$) dosage treatment groups were each composed of two separate plots, one of which was sprayed three and the other four times, with an interval of 7 days between sprays. The wheat plant, soil as well as matured or nearly matured wheat grain were sampled to determine final myclobutanil residues. The wheat and soil samples were harvested on days 20 and 30 post-spraying, respectively. In addition, control samples were obtained from the control plot. When they were analysed, all the field samples were based on dry weight.

The half-life of pesticide in different matrices was calculated using the first-order rate equation: $C_t = C_0 e^{-kt}$, where C_t is the concentration of the myclobutanil residue at time t; C_0 is the initial concentration ($t = 0$); and k is dissipation constant (days^{-1}). The half-lives ($t_{1/2}$) were determined from the k value for each experiment, being $t_{1/2} = \ln 2/k$.

2.4 *Soil extraction*

An aliquot (10 g) soil was weighed into a 150 mL conical flask and 30 mL of methanol was added. The mixture was shaken vigorously for 45 min, filtered and then washed by 20 mL methanol. The filtrate transferred to 500 mL separatory funnel, to which 80 mL water and 20 mL saturated NaCl were added, and extracted with 40×2 mL portions of dichloromethane. The combined extract was collected, dried over anhydrous Na_2SO_4 and concentrated to near dryness in a vacuum rotary evaporator at $35°C$. The residue obtained was dissolved in acetone (1 mL) for GC-ITMS analysis.

2.5 *Wheat plant and wheat grain extraction*

An aliquot (10 g) of sample was weighed into a 150 mL conical flask and extracted with 50 mL acetonitrile for 2 min using Ultra-Turrax T25 mixer at 5000 rpm. The mixture was then filtered to a 50 mL measuring cylinder equipped with glass stopper, to which 8 g NaCl was added. The mixture was shaken vigorously for 3 min. Finally, 25 mL of the organic layer was carefully evaporated to dryness with a rotary evaporator at $35°C$. The residue obtained was dissolved in *n*-hexane-acetone (4 : 1, v/v) for further clean-up. Extract purification was performed on Sep-Pak silica cartridges. Before use cartridges were conditioned with 5 ml *n*-hexane-acetone (4 : 1, v/v) without allowing the cartridges

to dry out. The cartridges were loaded with the extract, eluted with a volume (15 ml) of *n*-hexane-acetone (2:1, v/v) into a 25 mL round bottom flask and evaporated at 35°C to dryness on the rotary evaporator. The residue was finally re-dissolved in acetone (1 mL) and homogenised with vortex agitation for GC-ITMS analysis.

2.6 *Apparatus and chromatography*

A Thermo Trace Ultra gas chromatography system (Waltham, MA, USA) fitted with a AS 3000 autosampler, splitless injector and a DB-35ms capillary column (30 m × 0.25 mm i.d., 0.25 µm film thicknesses) supplied by J&W Scientific (Folsom, CA, USA) was used to determine myclobutanil. It was coupled to a POLARIS Q ion trap mass spectrometer and equipped with Xcalibur version 1.4.1 software. The injector was operated at 250°C and the pretreated sample volume of 1 µL was injected in the splitless GC-MS system. The oven was programmed as follows: 70°C (1 min), increased to 280°C (20°C min^{-1}) and held for 5 min. The ion trap mass spectrometer was operated in the electron ionisation (EI) mode at 70 eV, with 10 min solvent delay. The ion source was set at 250°C and the transfer line at 250°C. Helium (99.999% purity) was used as the carrier gas at constant flow rate of 1.0 mL min^{-1}. The target compound was identified and confirmed at full scan (*m/z* 50–400), and quantified in multiple reaction monitoring mode.

3. Results and discussion

3.1 *GC/ITMS analysis*

For myclobutanil, there were four possible precursor ions: *m/z* 179, 206, 245 and 288 from Figure 1. Although the ions at *m/z* 206, 245 and 288 were the best in terms of selectivity, they were discarded because of their very poor signal intensity and, in consequence, low sensitivity. In a compromise between sensitivity and selectivity, the ion at *m/z* 179 was chosen as the precursor ion. The next was acquiring a product ion scan over a range of excitation energies in order to choose the best product ions (Figure 2). From the product ion scan of *m/z* 179, the two product ions selected were *m/z* 125 and 152 with the excitation energy of 1.5 voltages (Figure 2).

3.2 *Method validation*

Figure 3 showed the chromatogram of the myclobutanil standard, blank and fortified sample of wheat plant. The retention time of myclobutanil was 12.4 min, and there were no interference peaks in this region of the chromatograph. In the condition of present work, the relative standard deviation of retention time (RT) in all the matrices analysed was never exceeding 1%. Quantification was performed by the external standard procedure. Linear calibration curves were obtained for the pesticide in different matrix at concentration range (0.002–10 mg L^{-1}). The correlation coefficients derived from the linear regressions were higher than 0.998 (Table 2), with strong correlation between concentration and area for target compound. Calibration was performed by external matrix-matched standards to eliminate the matrix effect and to obtain a more realistic determination.

The limit of detection (LOD) and the limit of quantification (LOQ) were defined as a signal-to-noise ratio (S/N) of 3:1 and 10:1, respectively. The LOD of target compound
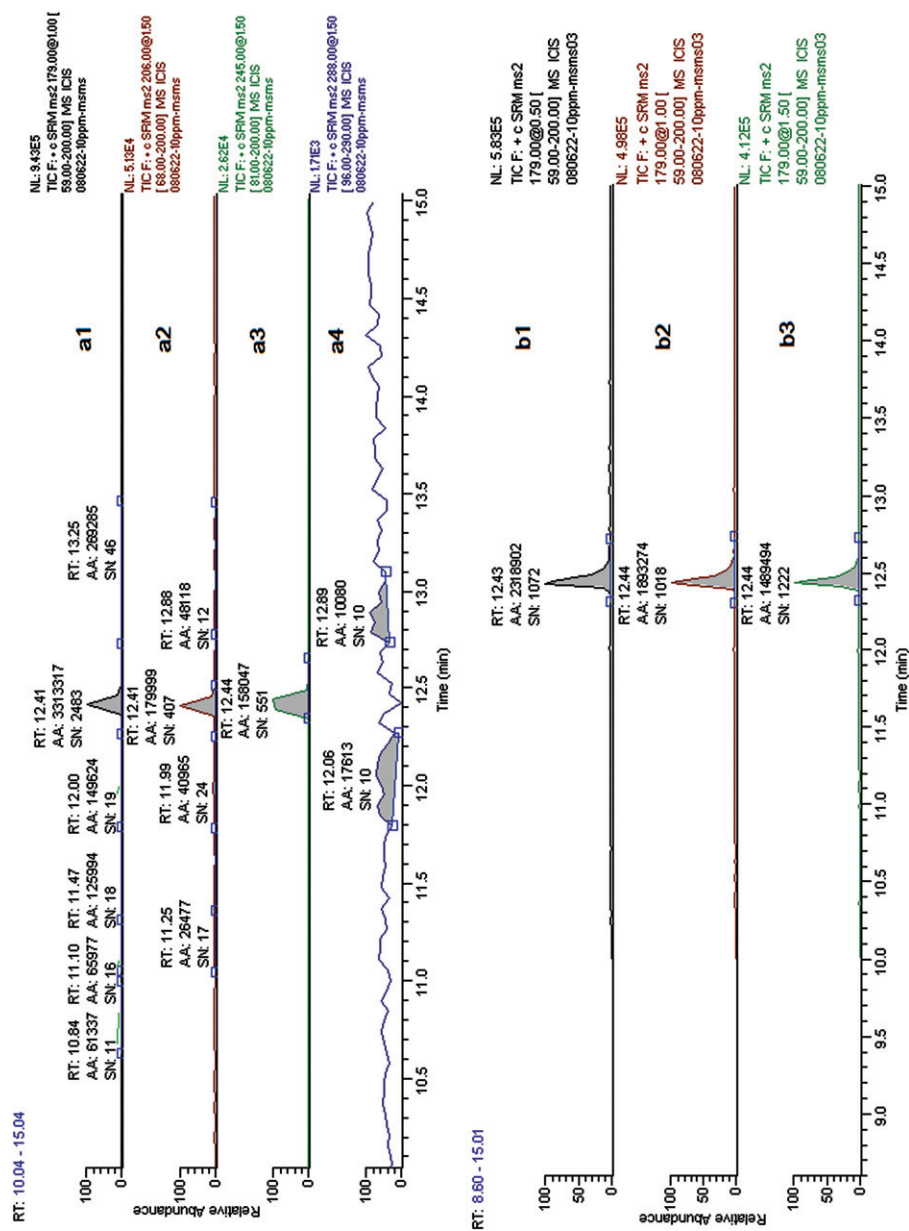
Figure 2. Optimisation of the MS/MS parameters for the pesticide myclobutanil: Selection of precursor ion: (a1) m/z 179, (a2) m/z 206, (a3) m/z 245, (a4) m/z 288. Precursor ion m/z 179 at the three excitation energies studied: (b1) 0.5 V, (b2) 1 V, (b3) 1.5 V.
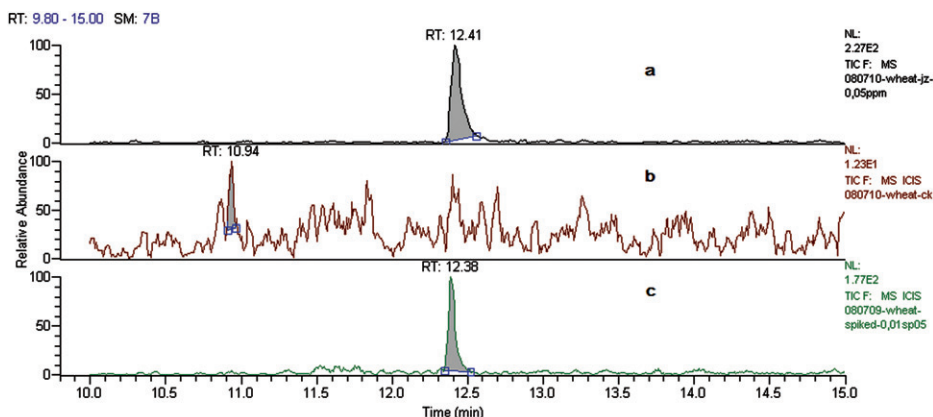
Figure 3. GC-ITMS (MS/MS) chromatograms at $50 \mu g \, L^{-1}$ (a), an untreated (b) and a fortified wheat plant sample at $10 \mu g \, kg^{-1}$ (c).

Table 2. Calibration equation, average recovery and relative standard deviation of myclobutanil at various matrices and levels.

	Wheat plant		Wheat grain		Soil	
	Mean[a]	RSD$_r$[b]	Mean[a]	RSD$_r$[b]	Mean[a]	RSD$_r$[c]
Calibration equation (0.002–10 mg L^{-1})	$Y = 26946C + 136$		$Y = 30794C + 483$		$Y = 31322C - 613$	
$R^2 (n = 3)$	0.9994		0.9998		0.9985	
Spiking levels (mg kg^{-1})	Mean[a]	RSD$_r$[b]	Mean[a]	RSD$_r$[b]	Mean[a]	RSD$_r$[c]
0.002	–[c]	–	–	–	83.6	19.7
0.005	–	–	84.9	17.3	–	–
0.01	97.7	7.0	82.6	10.0	75.0	13.8
0.1	76.4	15.9	88.1	12.4	94.4	12.6
0.5	78.8	11.4	–	–	93.4	3.0
10	87.3	6.3	92.6	3.8	88.7	4.1

Notes: [a]$n = 5$ mean of recovery.
[b]RSD, relative standard deviations.
[c]Do not test at this level.

was $0.6 \mu g \, kg^{-1}$ in GC-ITMS (MS/MS mode), and the LOQ was $2 \mu g \, kg^{-1}$ in different matrices. This limit was below the maximum residue limit (MRL, $0.02 \, mg \, kg^{-1}$) established in wheat by EU for myclobutanil.

The recovery, accuracy and the precision studies were evaluated by extraction and analysis of five replicates using the proposed described method for wheat plant, wheat grain and soil matrices (Table 2). The recoveries in wheat plant were varied from 76.4 to 97.7% with RSD from 6.3 to 15.9% at four spiking levels ($0.01, 0.1, 0.5, 10 \, mg \, kg^{-1}$); that in wheat grain were varied from 82.6 to 92.6% with RSD from 3.8 to 17.3% at four spiking levels ($0.005, 0.01, 0.1, 10 \, mg \, kg^{-1}$); and that in soil were from 75.0 to 94.4% with RSD from 3.0 to 19.7% at five spiking levels ($0.002, 0.01, 0.1, 0.5, 10 \, mg \, kg^{-1}$). The mean recovery was in excess of 85.5% in all cases, which were in the range expected for residue

Table 3. Dissipation of myclobutanil residues in soil and wheat plants grown in Beijing, Shandong and Henan, China.

Sampling time (days)	Shandong		Beijing		Henan	
	Residue $(mg\,kg^{-1})$	Dissipation (%)	Residue $(mg\,kg^{-1})$	Dissipation (%)	Residue $(mg\,kg^{-1})$	Dissipation (%)
Soil						
0	1.280 ± 0.200	0.0	1.930 ± 0.225	0.0	2.002 ± 0.420	0.0
1	1.217 ± 0.160	4.9	1.537 ± 0.197	20.4	1.616 ± 0.200	19.3
3	1.088 ± 0.090	15.0	1.227 ± 0.085	36.4	1.339 ± 0.300	33.1
7	0.932 ± 0.070	27.2	1.043 ± 0.104	46.0	1.043 ± 0.050	47.9
14	0.613 ± 0.032	52.1	0.737 ± 0.190	61.8	0.816 ± 0.250	59.2
21	0.586 ± 0.050	54.2	0.463 ± 0.074	76.0	0.381 ± 0.130	81.0
28	0.165 ± 0.021	87.1	0.197 ± 0.038	89.8	0.161 ± 0.040	92.0
35	0.064 ± 0.020	95.0	0.083 ± 0.021	95.7	0.048 ± 0.006	97.6
42	0.040 ± 0.006	96.8	0.030 ± 0.009	98.4	0.048 ± 0.030	97.6
60	0.012 ± 0.007	99.0	0.017 ± 0.003	99.1	0.027 ± 0.012	98.6
90	0.011 ± 0.003	99.1	ND	—	0.017 ± 0.008	99.2
Half-life ($t_{1/2}$)	11.1 days		11.0 days		11.7 days	
Wheat plant						
0	7.665 ± 1.200	0.0	8.079 ± 1.800	0.0	10.40 ± 1.700	0.0
1	4.257 ± 0.600	44.5	3.806 ± 1.100	52.9	8.390 ± 1.400	19.3
3	1.131 ± 0.200	85.2	0.674 ± 0.140	91.7	4.654 ± 0.900	55.2
7	0.166 ± 0.045	97.8	0.632 ± 0.210	92.2	2.391 ± 0.620	77.0
14	0.035 ± 0.012	99.5	0.515 ± 0.080	93.6	0.958 ± 0.130	90.8
21	0.008 ± 0.004	99.9	0.300 ± 0.033	96.3	0.445 ± 0.200	95.7
28	0.010 ± 0.001	99.9	0.101 ± 0.030	98.8	0.046 ± 0.015	99.6
35	0.003 ± 0.001	100.0	0.006 ± 0.002	99.9	0.042 ± 0.020	99.6
Half-life ($t_{1/2}$)	3.5 days		4.5 days		4.3 days	

Notes: These data were based on 2 times the recommended dosage of myclobutanil; ND: not detected.

analysis according to the agricultural professional standard of the People's Republic of China (NY788-2004).

3.3 Dissipation of myclobutanil in wheat plant and soil under natural field conditions

Half-lives ($t_{1/2}$) and other statistical parameters of myclobutanil residue dissipation were calculated from the experimental data and summarised in Table 3. No residues of myclobutanil were detected in any analysed control wheat and soil sample. Under the natural field conditions the initial concentrations of myclobutanil in wheat plant were 7.67, 8.08 and 10.40 mg kg^{-1} in Shandong, Beijing and Henan at 2 times the recommended dosage, respectively. The myclobutanil residues of wheat plants were dissipated by more than 98% in 28 days at the 2 times recommended dosage in three fields of Shandong, Henan and Beijing. From Table 3, the half-lives were 3.5, 4.5 and 4.2 days in Shandong, Beijing and Henan, respectively. During the dissipation experimental period occasional rainfall (6–8 mm) was observed 6 days and 8 days after application in Shandong, but not in Beijing and Henan. Whereas the maximum day temperatures ranged from 13 to 33°C in Shandong, which was similar to that in Beijing and Henan which was in the range of

13–35 and 14–33°C, respectively. So the rainfall may be the important reason why myclobutanil dissipated more rapidly in wheat plant located in Shandong than that located in Henan and Beijing.

The initial concentrations of myclobutanil in soil from the Shandong, Henan and Beijing experimental field were found to be 1.28, 1.93 and 2.00 mg kg^{-1}, respectively. There was a gradual decrease in the myclobutanil content, and after 60 days, myclobutanil had dissipated to 0.012, 0.017 and 0.027 mg kg^{-1} in the three different districts. The half-life values calculated were 11.1, 11.0 and 11.7 days in Shandong, Beijing and Henan, respectively (Table 3). In the field, besides the effect of organic matter, CEC and pH on degradation of fungicide, soil texture might have played an important role in the degradation of myclobutanil residue. There were little differences of organic matter, CEC and pH from the information of three soils and soil collected from Beijing had less clay than that from Shandong and Henan. Fungicide should be dissipated faster in sandy loam than in clay loam. However, it was probably the rainfall that dissipated myclobutanil faster in Shandong than in Henan and similar to Beijing. Furthermore, the half-lives of myclobutanil at real conditions in this study were much shorter (3.5–4.5 days in wheat plant and 11.0–11.7 days in soil) than that from barley to malt (244 days) [25] and that during storage of the spent grains (82 days) [26].

3.4 *Residues of myclobutanil in wheat and soil*

The amount of myclobutanil detected in wheat grain sampled on days 20 and 30 post application did not exceed 0.016 mg kg^{-1}. By comparison, the maximum residue recorded in plant material was less than 0.53 mg kg^{-1}. However, myclobutanil residues in soil ranged from 0.01 to 0.83 mg kg^{-1} (Table 4).

In the dissipation of soil, the bare soil plot was used and the myclobutanil was sprayed into the soil surface directly. However, the fungicide was sprayed into the wheat plant mostly in the final residue study at the same dose. The fungicide residue in soil was only the dropping from plant or the sporadic spraying. So, the residue of myclobutanil from final residue study in soil (Table 4) was lower than that from the dissipation study (Table 3), even though the fungicide was applied 3–4 times. In addition, it can be found from Table 3 that the dissipation rate of myclobutanil in wheat plant was fast (the half-lives were 3.5–4.5 days). The concentrations became very little after 7 days when the fungicide was sprayed next time compared with that at time zero. On the other hand, the dissipation study was conducted at the early growth stage of wheat and the weight of the wheat plant was very little. This led to the concentration of myclobutanil being relatively high. But the wheat plant weight became larger and larger in the final residue study, which led to the small concentration of myclobutanil in wheat plant. The rapid dissipation rate and the growth dilution effect of wheat plant may be the factors that the residues in wheat plant in the final residue study (Table 4) were similar to or even lower than that in the dissipation study at 120 g a.i. ha^{-1} rate.

Although myclobutanil residues in soil did not vary as much among different areas, residues in grain and plant material were dramatically lower in Shandong and Henan than in Beijing. Residues in soil were higher than those detected in either wheat grain or plant material, and this may be the result of the fact that myclobutanil dissipated more slowly in soil than in plant material. Furthermore, because wheat grain was encapsulated with wheat hull, fungicide was sprayed to the surface of wheat plant rather than the grain and

Table 4. Final residues of myclobutanil in soil of experimental wheat fields, wheat plants and grain material grown in Shandong, Beijing and Henan, China.

Dosage (g a.i. ha^{-1})	Number of times sprayed	Sampling time (days)	Soil	Wheat plants	Wheat grain
Beijing					
60	3	20	0.20 ± 0.042	0.08 ± 0.0100	0.007 ± 0.001
		30	0.01 ± 0.002	0.011 ± 0.005	ND
	4	20	0.31 ± 0.015	0.12 ± 0.0045	0.012 ± 0.005
		30	0.20 ± 0.022	0.04 ± 0.0015	0.004 ± 0.002
120	3	20	0.49 ± 0.110	0.23 ± 0.0520	0.013 ± 0.001
		30	0.12 ± 0.025	0.14 ± 0.0210	0.004 ± 0.001
	4	20	0.83 ± 0.220	0.45 ± 0.0650	0.016 ± 0.003
		30	0.24 ± 0.070	0.19 ± 0.0820	0.011 ± 0.004
Shandong					
60	3	20	0.12 ± 0.04	0.06 ± 0.0150	0.004 ± 0.001
		30	0.05 ± 0.01	0.009 ± 0.002	ND
	4	20	0.12 ± 0.03	0.07 ± 0.0082	0.009 ± 0.004
		30	0.07 ± 0.02	0.02 ± 0.0042	0.001 ± 0.001
120	3	20	0.15 ± 0.09	0.15 ± 0.0400	0.005 ± 0.002
		30	0.09 ± 0.02	0.04 ± 0.0150	ND
	4	20	0.27 ± 0.09	0.25 ± 0.0800	0.015 ± 0.005
		30	0.13 ± 0.04	0.09 ± 0.0450	0.006 ± 0.002
Henan					
60	3	20	0.11 ± 0.020	0.02 ± 0.003	ND
		30	0.02 ± 0.005	ND	ND
	4	20	0.12 ± 0.005	0.05 ± 0.010	0.001 ± 0.001
		30	0.06 ± 0.031	ND	ND
120	3	20	0.26 ± 0.082	0.12 ± 0.020	ND
		30	0.15 ± 0.050	0.03 ± 0.005	ND
	4	20	0.31 ± 0.025	0.53 ± 0.060	ND
		30	0.16 ± 0.042	0.08 ± 0.045	ND

Note: The interval was 7 days between sprays; ND: not detected.

the myclotubanil that transport from wheat plant to grain was very little, the fungicide residue in wheat plant material were higher than that in grain in all the three districts. On the other hand, comparatively low residues in wheat grain suggested that this pesticide may otherwise cause no problems in terms of food safety.

4. Conclusions

From the results obtained it can be concluded that the proposed method is suitable for quantification of myclobutanil residue in wheat and soil matrix. This method appears to be suitable for routine monitoring of myclobutanil residue in wheat and soil as the results show good recovery with low coefficients of variation. The dissipation rate study of myclobutanil in wheat plant and soil showed that this agricultural chemical was easily degradable. The data also showed myclobutanil residue values measured in wheat grain were clearly below the MRL value of myclobutanil in EU ($0.02 \, \text{mg kg}^{-1}$) with the interval of 20 days at the recommend dosage after last application. According to the results of this

study, wheat crop should be safe with myclobutanil (EC, 25%) not more than 3 times at the recommend dosage and with an interval of at least 7 days between each application.

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