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Short Communication

Dissipation and Residue of Picoxystrobin in Banana and Soil under Field Conditions

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Abstract

The dissipation and final residues of picoxystrobin in banana and soil were determined by gas chromatography equipped with an electron capture detector (GC-ECD). The dissipation half-lives of picoxystrobin were 10.7-12.1 days in banana, and 12.5-13.4 days in soil at Nanning (Guangxi) and Zhanjiang (Guangdong). The final residues of picoxystrobin in banana and soil were determined after the third and fourth applications at recommended dose and 1.5 times recommended dose, respectively. Picoxystrobin residues in banana 28 days after the last treatment were below 0.686 mg/kg, in banana sarcocarp below 0.159 mg/kg, and in soil below 0.227 mg/kg.

Keywords: picoxystrobin, dissipation, residues, banana, soil

Introduction

Picoxystrobin (methyl (2)-3-methoxy-2-{2-[6-(trifluoromethyl)-2-pyridyloxymethyl] phenyl} acrylate) belongs to the group of strobilurin fungicides that are derivatives from the naturally occurring β-methoxyacrylates. The mechanism of action is the inhibition of mitochondrial respiration by binding to the Q_0 site of cytochrome b, which eventually leads to the disruption of the energy cycle [1, 2]. Because of its powerful biological activity, picoxystrobin was registered in numerous crops for controlling or suppressing a broad spectrum of diseases. Banana is one of the major fruits cultivated in China. Sigatoka disease is recognized as one of the most destructive diseases of banana. Fungicides are employed to protect banana from sigatoka disease in production. The use of synthetic fungicides is common in conventional agriculture and poses a risk to humans and the environment [3].

Recently, DuPont Crop Protection has also registered picoxystrobin in bananas of China. With extensive and intensive use, picoxystrobin might cause food contamination and is a potential threat to human health. To assess the risk, ensure the safety of food for consumers, and regulate international trade and legislation, the regulatory authorities of many countries have established maximum residue limits (MRLs) for picoxystrobin in different commodities. European Regulation No. 396/2005 (2005) has established the MRL for picoxystrobin in products of plant origin [4]. The MRL of picoxystrobin for barley grain is 0.2 mg/kg in European Union and New Zealand, and 0.3 mg/kg in the United States and Canada (FAS International Maximun Residue Level Database, http://www.mrldatabase.com). China has not stipulated maximum residue levels (MRLs) for picoxystrobin in food. At present, many reports focus on diverse analytical methodologies for determining picoxystrobin residues in food samples. The most commonly used ways are based on liquid or gas chromatography coupled to mass spectrometry detectors with different sample preparation methods [5, 6]. Electroanalytical and

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enzyme-linked immunosorbent assays are described for the determination of picoxystrobin [8-11]. Flow injection analysis with chemiluminescence detection has also been used for the determination of picoxystrobin after ultrasound-assisted extraction [12]. Stenrød et al. have evidenced the negative effects of picoxystrobin on soil microbial community structure and respiration activity [13]. Wang et al. studied the toxicity assessment of picoxystrobin to the epigeic earthworm Eisenia fetida, which showed potentially detrimental effects to soil fauna (earthworms) [14]. To our knowledge, limited work was reported about the residue and dissipation of picoxystrobin under field conditions.

Research evaluated the dissipation rate of picoxystrobin under field conditions, which can help establish the maximum residue level value and determine pre-harvest interval.

Experimental

Picoxystrobin standard (99.9%, purity) and 250 g/l SC (Acanto SC) were provided by DuPont Trading Co., Ltd (Shanghai, China). Methanol of chromatographic grade was purchased from TEDIA (USA). Neutral alumina (100-200 mesh) was obtained by Sinopharm Chemical Reagent Co., Ltd (Shanghai, China), activated by heating at 550ºC for 4 h and deactivated with 5% deionized water prior to use. All chemicals used in this study are of analytical grade. Other solvents also were of analytical grade and distilled before use.

Field experiments, including the dissipation and final residue treatment, were conducted at Zhanjiang (Guangdong) and Nanning (Guangxi) in China during summer 2009-10, according to NY/T 788-2004 (Guideline on Pesticide Residue Trials) issued by the Ministry of Agriculture, the People's Republic of China. Each treatment consisted of one control and three replicates of treated samples with an area of 30 m^2 in the field experiment for residue and dissipation. The untreated banana plot and soil plot were sprayed with the same amount of water for control treatment.

To evaluate the dissipation dynamic of picoxystrobin in banana and soil, picoxystrobin (250 g/l SC) was dissolved in water and applied onto the surface of banana at 2 times recommended dose of 250 g a.i./hm^2 and the bare soil with no plants at 2.5 times recommended dose of 312.5 g a.i./hm^2. About 2 kg banana samples and representative 2 kg surface soil (0-10 cm depth) samples were collected randomly from several points in each plot at 2 h (calculated as the original concentration), 1, 3, 5, 7, 14, 21, 28, 35, 42, and 49 days after application. To investigate the final residue of picoxystrobin in banana and soil, two doses (recommended dose 125 g a.i./hm^2 and 1.5 times recommended dose 187.5 g a.i./hm^2, with two treatments: spray 3 times and 4 times) were applied to separate plots with interval of 7 days between each application, respectively. Both banana and soil samples were collected at 14, 21, and 28 days before harvest. All the samples were stored in a deep freezer at -20ºC until further analysis.

The banana samples were homogenized thoroughly in a blender. The soil sample was prepared by removing any large stones, drying in the shade and sieving through a 40 mesh. Banana, sarcocarp and soil samples (20 g) were extracted with 80 ml methanol by a mechanical shaker for 30 min. The overlayer was filtered through a 12-cm Buchner funnel, and then washed with methanol (20 ml). The organic layers were combined and evaporated to about 30 ml with rotary vacuum evaporation, then concentrated solution was transferred to 250 ml separating funnel containing 4% sodium chloride solution (70 ml), and partitioned, respectively, with ethyl acetate three times (50, 30, 30 ml). The ethyl acetate phases were combined, dehydrated, and concentrated to dryness for further cleanup with column chromatography.

The column (10 cm length × 20 mm i.d.) was packed with 2 cm of anhydrous sodium sulfate at the bottom and covered with neutral alumina (3.0 g) and another 2 cm of anhydrous sodium sulfate were placed above the neutral alumina at the top of the column. The column was firstly prewetted with petroleum ether (20 ml). After the dry extract was dissolved in 5 ml of petroleum ether/ethyl acetate (100:2 V/V) and transferred completely to the column, the column was eluted with ethyl acetate (50 ml). The eluate was evaporated to about 2 ml with a rotary vacuum evaporator at 50ºC. The residues were dissolved in 1 ml of acetonitrile and analyzed by GC-ECD.

The samples were analyzed by GC-ECD (Agilent 7890, USA). The column was HP-5 (30 m length × 0.25 mm i.d. × 0.32 mm film thickness, J&WScientific, USA) used for picoxystrobin determination. The instrument conditions are as follows: inlet temperature 250ºC, initial oven temperature 120ºC for 1 min, then increased to 260ºC with 20ºC/min rate and held for 8 min. Ultra-pure nitrogen was used as a carrier gas and injections were carried out in splitless mode using 1 μl injection volume.

The degradation process of the picoxystrobin in banana and soil were calculated according to Equation

$$C_t = C_0 e^{-kt}$$

...where t is the time (days) after picoxystrobin application, C_t is the concentration of the picoxystrobin residue at the time of t, C_0 represents the initial deposits after application, and k is the degradation rate constant (d^{-1}). The half-life ($t_{1/2}$) is defined as the time required for the pesticide residue level falling to the half of the initial residue level after application and calculated using Equation ($t_{1/2} = \ln 2/k$) for each experiment.

Results and Discussion

The standard calibration curve of picoxystrobin during GC-ECD analysis was constructed by plotting the analyte concentration versus peak area. The calibration curve showed excellent linearity ($y = 23764x + 20.251$) in the concentration range of 0.1-1 mg/l with a correlation coeffi-

Table 1. Recovery and RSD of picoxystrobin at different samples.

Sample	Spiked levels (mg/kg)	Recovery (%)					Average (%)	RSD (%)
		1	2	3	4	5		
Soil	0.02	99.51	95.48	97.40	95.82	90.14	95.67	3.64
	0.2	102.21	92.14	92.75	94.93	87.61	93.93	5.69
	2.0	86.69	80.83	94.26	94.04	84.82	88.04	6.85
Sarcocarp	0.02	97.44	92.44	95.43	93.81	95.09	94.84	1.97
	0.2	87.99	106.41	98.60	96.16	102.93	98.84	7.15
	2.0	86.11	96.24	91.49	81.16	79.88	86.97	7.95
Banana	0.02	91.12	104.52	96.94	86.10	98.12	95.36	7.37
	0.2	100.08	86.10	92.72	103.49	89.07	94.33	7.72
	2.0	89.74	87.87	87.33	87.94	87.67	88.11	1.07

cient of $R^2 = 0.9972$. The limit of quantification (LOQ) was established at 0.01 mg/kg, which yielded a signal-to-noise (S/N) ratio of 10. The limit of detection (LOD) was 0.003 mg/kg at a signal-to-noise ratio of 3. To validate and evaluate the accuracy of the method, recoveries of picoxystrobin in different substrates were determined at three different fortification levels (Table 1). The mean recoveries were in the range of 86.97-98.84% for the three matrices. The relative standard deviations (RSDs) for repeatability ranged from 1.07% to 7.95%, which is within the acceptable limits for routine analysis of picoxystrobin residues. The results indicated that the approach was suitable for the determination of picoxystrobin residues in banana, sarcocarp, and soil.

Fig. 1 showed the dissipation dynamics of picoxystrobin in banana from two different locations. The initial concentrations of picoxystrobin in banana were 2.315 mg/kg in Guangxi and 2.289 mg/kg in Guangdong. As can be seen in Fig. 1, the largest amount of picoxystrobin in banana was rapidly dissipated within the first week after application. The concentrations of picoxystrobin residues were less than 0.2 mg/kg in 7 weeks after application at Nanning and Zhanjiang. The half-lives of picoxystrobin in banana were 12.1 and 10.7 days for Nanning and Zhanjiang locations, respectively. The decline of picoxystrobin residues was faster at Zhanjiang than Nanning. The result may be due to the frequent rainfall at Zhangjiang. According to record, the rainfall amounts during the period of field trial were 1,360 mm at Nanning and 1,849 mm at Zhanjiang, respectively. Seenivasan and Muraleedharan maintained that treated crop growth dilution might play an important role in the decline of pesticides in crops [15]. However, the banana growth had a limited contribution to the picoxystrobin dissipation rate, because a rapid dissipation appeared mostly within the first two weeks after application. These results are similar to some reports for other pesticides in many crops [16, 17].

The persistence of picoxystrobin residues in soil is presented in Fig. 2. The maximum concentration of picoxystrobin in soil was 0.413 mg/kg and 0.631 mg/kg in 1 day after application at Nanning and Zhangjiang, respectivity. At six weeks after application, the concentration of picoxy-

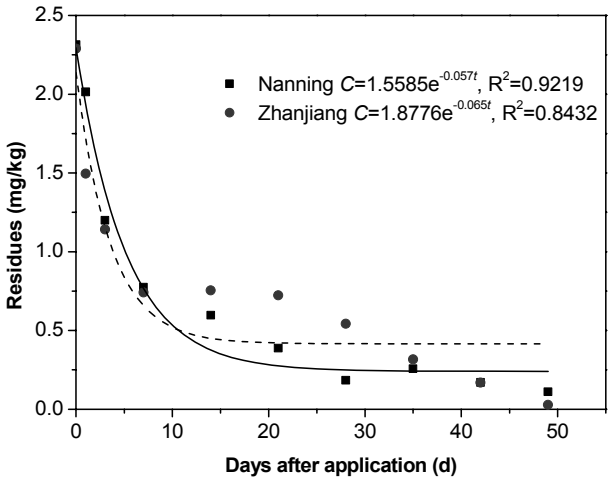

Fig. 1. Dissipation dynamics of picoxystrobin in banana at Nanning and Zhanjiang.

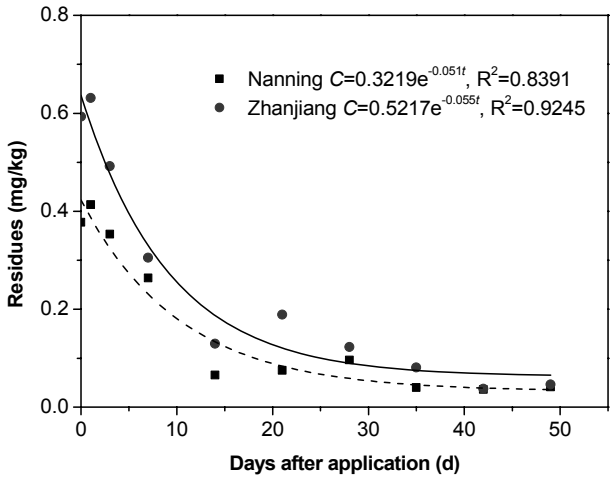

Fig. 2. Dissipation dynamics of picoxystrobin in soil at Nanning and Zhanjiang.

Table 2. The final residue of picoxystrobin in banana, sarcocarp, and soil (2009).

Dose (g a.i./hm^2)	Interval (days)	Spray times	Nanning			Zhanjiang		
			Banana	Sarcocarp	Soil	Banana	Sarcocarp	Soil
125	28	3	0.174	0.055	<0.01	0.110	0.057	<0.01
		4	0.651	0.095	0.031	0.333	0.110	0.061
	21	3	0.168	0.032	<0.01	0.144	0.087	0.020
		4	0.731	0.091	0.031	0.217	0.062	0.204
	14	3	0.260	0.039	0.162	0.289	0.113	0.024
		4	0.932	0.091	0.129	0.317	0.121	0.091
187.5	28	3	0.686	0.086	<0.01	0.224	0.110	0.040
		4	0.613	0.092	<0.01	0.468	0.159	0.046
	21	3	0.963	0.105	0.010	0.272	0.104	0.038
		4	1.049	0.103	0.016	0.119	0.131	0.055
	14	3	0.674	0.102	0.139	0.396	0.105	0.051
		4	0.869	0.098	0.099	0.233	0.117	0.046

Table 3. The final residue of picoxystrobin in banana, sarcocarp, and soil (2010).

Dose (g a.i./hm^2)	Interval (days)	Spray times	Nanning			Zhanjiang		
			Banana	Sarcocarp	Soil	Banana	Sarcocarp	Soil
125	28	3	0.269	0.059	0.034	0.074	0.013	<0.01
		4	0.451	0.077	0.026	0.019	0.012	0.037
	21	3	0.325	0.073	0.167	0.046	0.016	0.046
		4	0.530	0.068	0.037	0.046	0.010	0.043
	14	3	0.324	0.070	0.124	0.460	0.018	0.043
		4	0.485	0.089	0.036	0.126	0.017	0.067
187.5	28	3	0.292	0.070	0.191	<0.01	<0.01	<0.01
		4	0.660	0.060	0.227	0.038	0.010	<0.01
	21	3	0.396	0.074	0.332	0.041	0.018	0.030
		4	0.697	0.090	0.159	0.040	0.018	0.120
	14	3	0.607	0.076	0.326	0.123	0.011	0.046
		4	1.037	0.115	0.087	0.081	0.017	0.041

strobin residues was reduced by 90.53% at Nanning and by 93.72% at Zhanjiang. The half-lives of picoxystrobin in soil were 13.4 and 12.5 days in Guangxi and Guangdong, respectively. In this study, different soil properties and climate at Nanning and Zhanjiang may cause different half-lives of picoxystrobin in soil. The result indicated that climate and soil properties were major factors on the dissipation of picoxystrobin in soil.

In the residue experiments, the solution of 250 g/l Acanto SC was sprayed directly on the banana tree and not on the soil with two different dosages and frequencies. The final residue of picoxystrobin after pre-harvest interval of 14, 21, and 28 days were shown in Tables 2 and 3. Picoxystrobin residues in banana were 0.081-1.037 mg/kg at day 14, 0.040-1.049 mg/kg at day 21, and below 0.686 mg/kg at day 28 after application. In 2010, picoxystrobin residues in sarcocarp were below 0.05 mg/kg in all treatments at Zhanjiang, but exceeded 0.05 mg/kg in all treatments at Nanning. At 28 days after the last spraying, picoxystrobin residues in sarcocarp were below 0.1 mg/kg

in all treatments (125 g a.i./hm^2 with three applications at a 7-day interval). The residue in soil was 0.024-0.326 mg/kg at day 14, below 0.332 mg/kg at day 21, and below 0.227 mg/kg at day 28. Up to now, no country has established the MRL for picoxystrobin in banana. This work would also be helpful for the Chinese government to establish the MRL of picoxystrobin in banana and to provide guidance on the proper and safe use of this fungicide.

Conclusion

In this paper, dissipation and residue of picoxystrobin in banana and soil were studied under field conditions. The results showed that the half-lives of picoxystrobin in banana were similar to that in soil. The final residue in banana and sarcocarp at a pre-harvest interval of 28 days was below 0.269 and 0.057 mg/kg with three applications at recommended dose, respectively. These results would provide guidance for setting the MRL of picoxystrobin on banana in China.

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