JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Metabolism of Strobilurins by Wheat Cell Suspension Cultures

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Supporting Information

ABSTRACT: Strobilurin fungicides are a leading class of antifungal chemicals used today in agricultural applications. Although degradation of some strobilurin fungicides has been assessed in plant residues, little information has appeared in the literature concerning the rates of metabolism of these fungicides in plants. In this study, we explored plant metabolism of three strobilurin fungicides, azoxystrobin, kresoxim-methyl, and trifloxystrobin, using wheat cell suspension cultures. Trifloxystrobin and kresoxim-methyl were completely metabolized within 24 h, whereas the metabolism of azoxystrobin was relatively slow with half-lives up to 48 h depending on specific experimental conditions. Metabolic rates of these fungicides were affected by the amounts of compound and cells added to the media. Structural analysis of metabolites of trifloxystrobin and kresoxim-methyl by high-resolution mass spectrometry (HRMS) and nuclear magnetic resonance spectroscopy (NMR) indicated that trifloxystrobin was first demethylated followed by subsequent hydroxylation, whereas kresoxim-methyl was largely demethylated. In contrast, a number of minor metabolites of azoxystrobin were present suggesting a differential metabolism of strobilurins by wheat cells.

KEYWORDS: *strobilurins, azoxystrobin, kresoxim-methyl, trifloxystrobin, wheat cell, metabolism, degradation*

INTRODUCTION

Strobilurins are derived from natural products isolated from basidiomycete fungi that include strobilurin A and oudemansin A produced by *Strobilurus tenacellus* and *Oudemansiella mucida*.[1] The strobilurins are known to reversibly bind to the quinol oxidation (Q_o) site of cytochrome b, a part of cytochrome bc_1 complex, located in the inner mitochondrial membrane of fungi and subsequently block electron transfer between cytochrome b and cytochrome c_1.[1,2] The interference in electron transfer leads to inhibition of mitochondrial respiration via ATP synthesis disruption. Since 1996, a number of synthetic strobilurin fungicides, including azoxystrobin, kresoxim-methyl, and trifloxystrobin (Figure 1) have been commercialized and widely used to control many fungal pathogens, particularly prior to infection or in the early development.[2]

Plant metabolism of fungicides is one of the important factors determining their biotic stability. In addition, metabolism of fungicides by plants can often lead to loss of fungicidal activities if degradation occurs at a certain chemical structure within the pharmacophore. Despite the importance of metabolism, information on plant metabolism of these strobilurins is limited.[1,2] Previously, long-term residual analyses of strobilurins in plants were conducted only under field conditions and showed that strobilurins can be metabolized to many metabolites in plants.[2−5] In these studies, carboxylic ester hydrolysis to the carboxylic acid was regarded as an important degradation route in plant metabolism. However, quantitative metabolic rates of different strobilurins in plants were not clearly described in the literature leading us to determine the comparative rates of metabolism of strobilurins in plant.

Plant cell cultures have been used as model systems to investigate the metabolic stability of xenobiotics in whole plants mainly because metabolism of xenobiotics in plant cell cultures resembles that in whole plants and the cell cultures can be more easily manipulated than whole plants.[6−8] In our study, we report the metabolism of three strobilurins, azoxystrobin, kresoxim-methyl, and trifloxystrobin in wheat cell suspension cultures with the determination of the rates of metabolism at different application rates and cell densities and the structural analyses of metabolites.

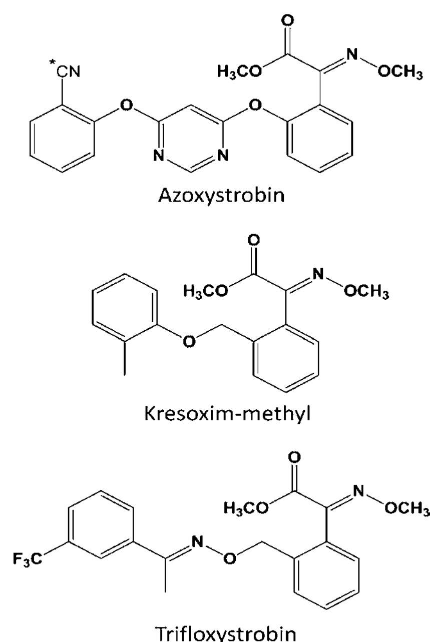

Figure 1. Chemical structures of three strobilurins used in this study, azoxystrobin, kresoxim-methyl, and trifloxystrobin. Asterisk (*) indicates the ^{14}C-labeled carbon position in the case of radiolabeled azoxystrobin.

Received: October 17, 2012
Revised: December 5, 2012
Accepted: December 6, 2012
Published: December 6, 2012

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■ MATERIALS AND METHODS

Strobilurins. Azoxystrobin, kresoxim-methyl, and trifloxystrobin were purchased from ChemService Inc. (West Chester, PA) and the ^{14}C-labeled azoxystrobin (23.13 mCi/mmol at 57.4 μCi/mL) was obtained from the Specialty Synthesis Group within Dow AgroSciences (Figure 1).

Wheat Cell Cultures and Strobilurin Treatments. Cell suspension cultures of wheat (*Triticum aestivum* L. cv. 'Anza') were prepared and grown as previously described.[8] Stock solutions of azoxystrobin, kresoxim-methyl, and trifloxystrobin were prepared at 1 mg/mL DMSO and added to 7 mL Murashige and Skoog media containing 2 mg/L 2,4-D (MS2D media) with or without 30 mg wheat cells at final concentrations of 0.1, 0.5, 1.0, 3.0, and 5.0 μg/mL media. Additional metabolism studies were performed using cell densities of 5, 10, 20, and 30 mg cells/mL media, where the cell cultures were treated with 5.0 μg of each strobilurin. Cell suspension cultures or culture media alone in 6-well culture plates were incubated at 130 rpm, 27 °C for 48 h on an orbital shaker (New Brunswick Scientific). To address potential phytotoxicity, cell viability was assessed during the incubation time using fluorescein diacetate, as previously described.[8] All of the metabolism experiments were performed using three replicates.

Extraction and Determination of Strobilurins and Their Metabolites in Wheat Cell Cultures. For the analysis of nonradiolabeled strobilurins, samples treated with azoxystrobin were collected at 0, 4, 24, and 48 h after treatment (HAT), whereas samples treated with kresoxim-methyl and trifloxystrobin were collected at 0, 2, 4, 6, 12, 24, and 48 HAT, depending on experimental conditions. Samples were transferred to 15 mL test tubes and mixed with 3.5 mL ethyl acetate by vigorously vortexing for 30 s, and the ethyl acetate fractions were collected. The extraction was repeated three times, and the resulting ethyl acetate fractions were combined and evaporated under N_2 gas flow. The dried samples were resuspended in 1 mL acetonitrile and the resuspended samples were analyzed by an Agilent-HP 1100 HPLC system with a photodiode array (PDA) detector and an electrospray ionization-mass spectrometry (ESI-MS) detector. Elution was carried out with initial gradient at 35:65 (v/v) of acetonitrile/water containing 1% acetic acid, followed by an increase in acetonitrile with a linear gradient to 100:0 (v/v) for 13 min, and returned to the initial condition at 35:65 (v/v) for 2 min. The HPLC analyses were performed on a 5 μm C_{18} reversed-phase column (Phenomenex Luna, 250 × 2.0 mm) at a flow rate of 1 mL/min. The peak area at 254 nm of each compound was used to quantify the recovery of the compound in samples. Equation, $C_t = C_0 e^{-\lambda t}$, where C_t and C_0 are quantities at t h and 0 h, respectively was used to describe the decay constant (λ). Decay constants of these compounds were determined after subtracting hydrolytic degradation in controls (no cells) from total degradation in treatments.

For the analysis of radiolabeled azoxystrobin, samples were collected at 0, 24, and 48 HAT. After following the extraction procedures described above, radioactivity in the resulting aqueous and ethyl acetate fractions was measured using a Beckman LS600 scintillation counter. The organic fractions were further analyzed using a Waters 2690 HPLC system with a Packard flow scintillation analyzer using the same HPLC conditions as described above.

Identification of Kresoxim-Methyl Metabolite M1 and Trifloxystrobin Metabolites M2 and M3. To prepare the acid metabolites M1 of kresoxim-methyl and M2 of trifloxystrobin,[9,10] 1 mg of each compound was dissolved in 1 mL acetone that contained 50% 0.05 N NaOH in water and stirred for 2 h at room temperature. An aliquot (10 μL) of the samples was 10-fold diluted in acetonitrile and 2 μL of the diluted samples was injected for liquid chromatography/time-of-flight mass spectrometry (LC/TOF-MS) analysis. The LC/TOF-MS system consisted of Agilent 1200 HPLC system which was connected to a 6200 TOF mass spectrometer (Agilent Technologies, Santa Clara, CA), equipped with dual electrospray interface. The LC/TOF-MS analysis was performed on a reversed-phase Hypersil Gold-C_{18} analytical column of 50 × 2.1 mm and 1.9 μm particle size (Thermo). An aliquot (3 μL) of sample extract was injected in each run. Mobile phase consisted of 0.1% formic acid in water (A) and acetonitrile (B). The chromatographic method held the initial mobile phase composition (2% B) constant for 1 min, followed by a linear gradient to 100% B up to 15 min, and held at 100% B for 2 min. The gradient was then immediately dropped to 2% B over 0.1 min, and re-equilibrated for 2 min before the next injection. The flow rate was 0.4 mL/min. For HRMS, the following operation parameters were used in a positive mode: capillary voltage, 3200 V; nebulizer pressure, 45 psig; drying gas flow rate, 11 L/min; gas temperature, 350 °C; skimmer voltage, 60 V; octapole rf, 250 V; fragmentor voltage (in-source CID fragmentation), 150 V. The presence of targeted metabolites M1 and M2 was identified by matching the accurate mass and isotope fingerprint of the mass spectra with the theoretical values through the search by molecular formula function of the *MassHunter* software. A matching score higher than 90 out of 100 with peak area more than 100 000 counts was considered high confidence.

For production of a subsequent metabolite M3 of trifloxystrobin, 50 μL DMSO aliquots containing 500 μg trifloxystrobin were added into 100 mL wheat cell culture in 250 mL flasks prepared at 10 mg cells/mL MS2D media, which were maintained at 130 rpm, 27 °C. A total of twenty cultures were incubated and combined to obtain M3. After 24 h incubation, the cultures were shaken with ethyl acetate, and the ethyl acetate fractions were collected and evaporated using a rotary evaporator. The dried extract (1.0 g) was suspended twice in 10 mL methanol and sonicated for 10 min. The resulting mixture was centrifuged at 2500 *g* for 10 min and the methanol solution was combined and dried. The dried material (0.5 g) was resuspended in 3 mL methanol and centrifuged, and the resulting methanol-soluble material (237 mg) was dissolved in 200 μL methanol and subjected to preparative HPLC. The isolation of M3 was conducted on an Agilent 1200 gradient system using a preparative Phenomenex Lunar column (250 × 21.2 mm, 10 μm) at 20 mL/min. The column was eluted with isocratic 55% acetonitrile and 45% water containing 0.1% formic acid. The fractions corresponding to a peak around 8.7 min (250 nm) were collected and dried to obtain M3.

The isolated M3 metabolite (4.0 mg) was dissolved in d_4-methanol (Cambridge Isotope Laboratories) and NMR spectra were obtained on a Varian Inova 600 MHz NMR equipped with a 5 mm z-gradient inverse detection probe using the standard Varian pulse sequences. HSQC and HMBC spectra were obtained using adiabatic pulses. 1D NOE experiments were obtained with a mixing time of 700 ms.

RESULTS AND DISCUSSION

Degradation of Strobilurins by Wheat Cell Suspension Cultures. Recovery of azoxystrobin and kresoxim-methyl in MS2D media alone was more than 80% at all times without a significant decrease (Figure 2) indicating that these compounds are stable in the media for 48 h. In contrast, the recovery of trifloxystrobin was slightly reduced, with approximately 35% of the compound lost at 48 HAT (Figure 2) suggesting that trifloxystrobin is less stable in the media than azoxystrobin and kresoxim-methyl. The potential hydrolysis of trifloxystrobin was not further pursued.

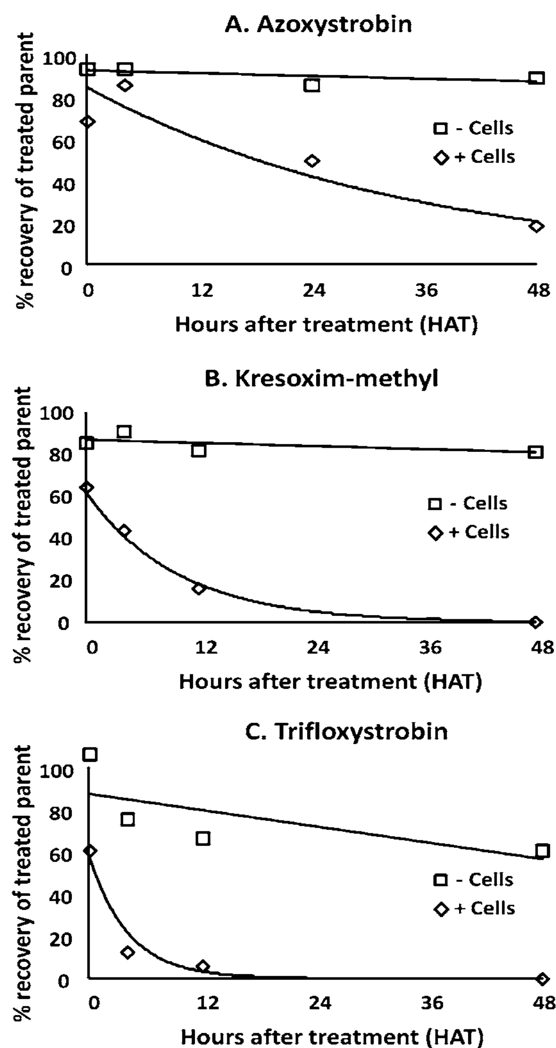


Figure 2. Degradation of azoxystrobin (A), kresoxim-methyl (B), and trifloxystrobin (C) at application rate of 5 μg/mL MS2D media in the absence (−) or presence (+) of wheat cells (30 mg/mL).

In the presence of wheat cells, azoxystrobin gradually degraded over time with a half-life about 24 h (Figure 2). For kresoxim-methyl, only 60% of the parent compound was present at 0 HAT, and the parent compound was not detectable at 48 HAT. About 60% of trifloxystrobin was also recovered at 0 HAT, 10% of trifloxystrobin at 4 h, and no trifloxystrobin was detected at 48 HAT.

Previously, it was shown that azoxystrobin was stable for 48 h and trifloxystrobin was still detectable after 48 h when the strobilurins were added to wheat cell cultures at the concentration of 20 μg/mL.[8] However, in our study, a precipitation of strobilurins in the media was observed when they were treated at concentrations higher than 10 μg/mL (data not shown). Considering the poor solubility of these strobilurins in water (<6 μg/mL), the slower degradation of strobilurins reported by Baloch et al.[8] may reflect the incomplete dissolution of strobilurins in the cell cultures.

Rates of Degradation of Strobilurins at Different Application Rates and Cell Densities. The effects of different application rates of azoxystrobin, kresoxim-methyl, and trifloxystrobin on rate of metabolism of these strobilurins by wheat cells were further investigated. As seen in Figure 3, the application rate of azoxystrobin had no significant effect on the rate of metabolism. On the contrary, the decay constant of kresoxim-methyl was increased as the application rate was decreased. The effect of application rate of trifloxystrobin on the rate of metabolism was somewhat inconsistent. Taken together, a negative correlation between degradation and application rates was found with kresoxim-methyl, but little effect of application rates on degradation was observed with azoxystrobin and trifloxystrobin. In addition, the rate of metabolism of azoxystrobin was slower than kresoxim-methyl and trifloxystrobin at all concentrations (Figure 3). The rate of metabolism of trifloxystrobin was faster than that of kresoxim-methyl at 5.0 μg/mL, as seen in Figure 2, but the rate of metabolism of trifloxystrobin was slower than that of kresoxim-methyl at the lower concentrations of 0.1, 0.5, and 1.0 μg/mL indicating that the application rate can affect the rate of metabolism.

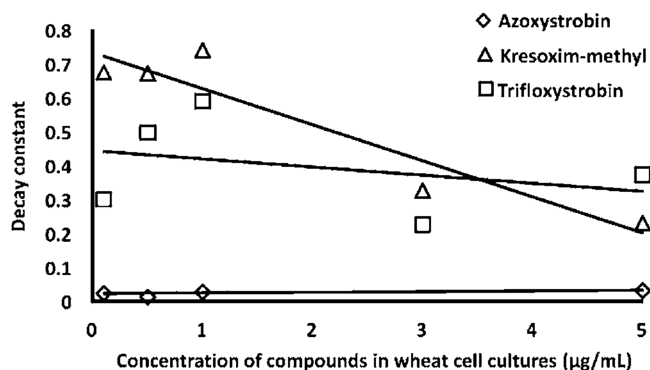


Figure 3. Decay constants of azoxystrobin, kresoxim-methyl, and trifloxystrobin calculated at application rates of 0.1, 0.5, 1.0, 3.0, and 5.0 μg/mL in the presence of wheat cells (30 mg/mL).

The rates of metabolism of these strobilurins were determined at different cell densities of 5, 10, 20, and 30 mg/mL at a constant application rate of 5.0 μg/mL. Because azoxystrobin was lethal to wheat cells at the lower cell densities of 5 and 10 mg/mL, the rates of metabolism of azoxystrobin were only obtained at cell densities of 20 and 30 mg/mL. There was little difference in decay constants of azoxystrobin between the two cell densities (data not shown), which ranged from 0.035 to 0.04. It is not certain whether the slower metabolic rate of azoxystrobin by wheat cells shown in this study relates to the phytotoxicity. Kresoxim-methyl and trifloxystrobin showed a positive correlation between cell concentration and decay constant, where the rate of metabolism decreased as the cell density increased (Figure 4). The linear correlations shown by kresoxim-methyl and trifloxystrobin were almost identical. Our results indicate that cell density can significantly affect the rates of metabolism of these strobilurins.

dx.doi.org/10.1021/jf304436j | J. Agric. Food Chem. 2013, 61, 47−52

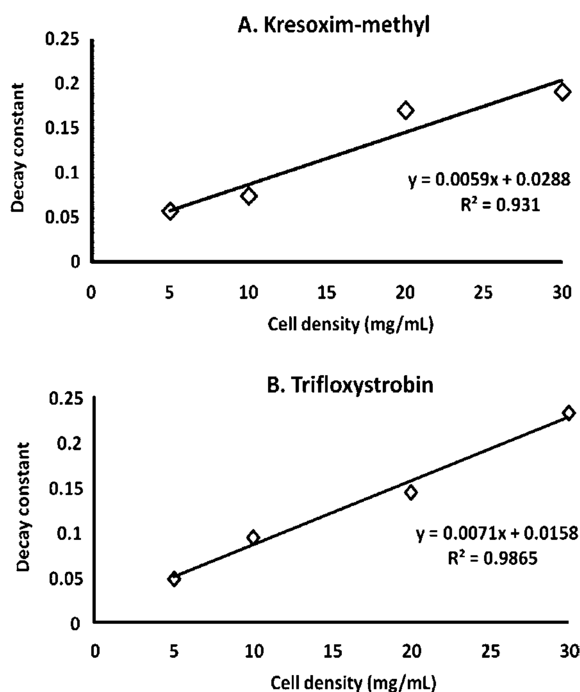


Figure 4. Decay constants of kresoxim-methyl (A) and trifloxystrobin (B) calculated at cell densities of wheat cells (5, 10, 20, and 30 mg/mL).

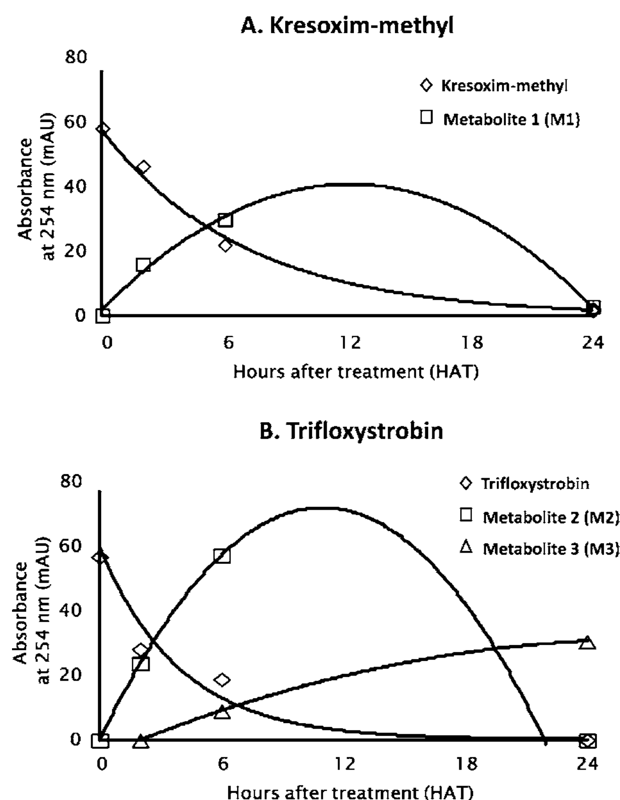


Figure 5. Metabolism of kresoxim-methyl to M1 (A) and trifloxystrobin to M2 and M3 (B). Compounds were applied to 7 mL wheat cell suspension cultures at the rate of 5.0 μg/mL in the presence of wheat cells (20 mg/mL).

It is generally anticipated that the increase in cell density or the decrease in application rate can enhance the rate of metabolism of xenobiotics because of the relatively increased abundance of cellular enzymes relative to amount of compound. In this regard, the rate of metabolism of kresoxim-methyl by wheat cells shown in Figures 3 and 4 was positively correlated with the ratio of cell mass to the amount of compound added. A similar pattern was also largely seen with trifloxystrobin, where the rate of metabolism of trifloxystrobin increased with the increased ratio of cell mass to the amount of compound (Figure 4). On the contrary, the ratio did not affect the rate of metabolism of azoxystrobin (Figure 3). Therefore, these results suggest that adding more wheat cells can increase the rates of metabolism of kresoxim-methyl and trifloxystrobin but will not increase the rate of metabolism of azoxystrobin

Metabolite Production of Kresoxim-Methyl and Trifloxystrobin in Wheat Cell Suspension Cultures. During the course of experiments, no major metabolite was found from samples treated with azoxystrobin (data not shown), whereas one major metabolite (M1) and two major metabolites (M2 and M3) were detected in extracts of cell cultures treated with kresoxim-methyl and trifloxystrobin, respectively (Figure 5). The M1 metabolite of kresoxim-methyl appeared at 2 HAT, continued to increase at 6 HAT, but disappeared at 24 HAT (part A of Figure 5). The production of M2 in cell cultures treated with trifloxystrobin was similar to that of M1 over time, but an additional metabolite M3 was found at 6 HAT and its production was 2-fold increased at 24 HAT (part B of Figure 5).

Identification of Kresoxim-Methyl Metabolite M1 and Trifloxystrobin Metabolites M2 and M3. Preliminary LC-MS analysis of M1 and M2 showed loss of 14 Da from the mass of kresoxim-methyl and trifloxystrobin respectively suggesting a demethylation of the parent compounds. Therefore, the demethylated products of kresoxim-methyl and trifloxystrobin were prepared by saponification and compared with M1 and M2 in wheat cell culture extracts using LC/TOF-MS to confirm whether M1 and M2 are the acid metabolites of kresoxim-methyl and trifloxystrobin.

As shown in parts A and B of Figure S1 of the Supporting Information, only one peak at 8.06 min bearing the predicted molecular formula $C_{17}H_{17}NO_4$ (accurate mass = 299.1156) of the demethylated kresoxim-methyl (M1) was observed at 2 h after saponification suggesting that the starting material underwent complete hydrolysis. The same peak with identical molecular formula and retention time was observed in the metabolism sample of kresoxim-methyl (parts C−E of Figure S1 of the Supporting Information). These data demonstrated that M1 was the demethylated metabolite of kresoxim-methyl.

Similarly, saponification of trifloxystrobin gave two peaks at 8.86 and 9.27 min corresponding to two peaks of the demethylated trifloxystrobin with a molecular formula of $C_{19}H_{17}F_2N_2O_4$ (parts A−C of Figure S2 of the Supporting Information) suggesting the starting material may be a mixture of two geometry isomers, as reported by Banerjee et al.[9] Indeed, LC/TOF-MS analysis of the starting material of trifloxystrobin showed two isomers that appeared at 9.98 and 10.45 min, with molecular formula $C_{20}H_{19}F_3N_2O_4$ (parts D−F of Figure S2 of the Supporting Information).

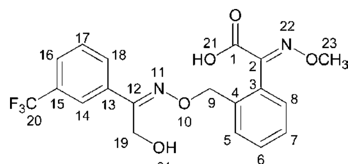
The LC/TOF-MS analysis of wheat cell culture treated with trifloxystrobin showed that the culture extract contained two sets of M2 peaks with retention time at 8.87 and 9.28 min (parts D and F of Figure S3 of the Supporting Information) and HRMS accurate masses of 394.1140 and 394.1142, and

these were identical to the corresponding demethylated trifloxystrobin products determined in saponification mixture (parts B and C of Figure S2 of the Supporting Information) demonstrating that M2 is demethylated metabolite of trifloxystrobin.

In addition, the culture extract contained two sets of M3 at 7.68 and 8.10 min (parts C and E of Figure S3 of the Supporting Information) with HRMS accurate masses of 410.1095 and 410.1089 respectively, and these masses were consistent with a molecular formula of $C_{19}H_{17}F_2N_2O_5$ that is possibly the hydroxylated M2. Because this type of structure of M3 has not been reported in the literature, the unknown M3 (major isomer at 8.10 min) was isolated from the wheat cell culture extract using a preparative HPLC and subjected to structure determination by NMR.

The proton and carbon NMR spectrum of the isolated metabolite M3 of trifloxystrobin in d_4-methanol are shown in Figures S4 and S5 of the Supporting Information respectively, and the ^1H NMR spectrum of trifloxystrobin is shown in Figure S6 of the Supporting Information. Assignments for the metabolite M3 are given in Table 1 and were based on chemical shifts, HSQC, and HMBC data. Key HMBC correlations are shown in Figure 6. Peaks corresponding to impurities from the culture extract were observed in both spectra of the metabolite. The presence of eight aryl protons in the metabolite M3 indicates that hydroxylation did not occur on either phenyl ring. The location of the hydroxyl group suggested by the mass spectrum was established by the absence of the methyl singlet of trifloxystrobin at 2.23 ppm and the appearance of a singlet (2H) at 4.74 ppm and a corresponding carbon resonance at 55.48 ppm for the metabolite M3 (Figures S4 and S6 of the Supporting Information) consistent with allylic hydroxylation of the methyl group of the oxime and is confirmed both by the observation of an HMBC correlation between the methylene protons at 4.73 ppm and the ipso carbon C-12 of the 3-trifluoromethyl-phenyl ring and by NOEs to the ortho phenyl protons (Figures S7−S10 of the Supporting Information). The data further support that the absence of the analogous hydroxylation of kresoxim-methyl was due to the absence of oximic methyl moiety in its structure. Therefore, M3 was identified as 19-hydroxyl trifloxystrobin acid.

Table 1. NMR Assignments for the Metabolite M3 of Trifloxystribin (600 MHz, d_4-Methanol)[a]



atom	^1H	^{13}C
1		169.48
2		158.36
3		136.54
4		134.84
5	7.43 (m)	129.70
6	7.33 (m)	129.24
7	7.33 (m)	128.66
8	7.18 (m)	129.74
9	5.18 (s)	76.73
12		158.36
13		137.10
14	7.97 (s)	125.04
15		131.65 (q)
16	7.64 (d, J = 7.8 Hz)	126.53
17	7.54 (t, J = 7.8 Hz)	130.11
18	7.93 (d, J = 7.8 Hz)	132.09
19	4.73 (s)	55.48
20		125.3(q)[b]
21	[c]	
23	3.87 (s)	62.77
24	[c]	

[a]Relative to CHD_2OD at 3.31 ppm and CD_3COD at 49.15 ppm. [b]Measured from the HMBC spectrum. [c]Not observed due to exchange with deuterium of the solvent.

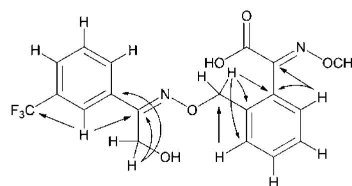


Figure 6. Key HMBC correlations identified in metabolite M3.

Metabolism of Azoxystrobin in Wheat Cell Suspension Cultures. Production of the acid metabolite of azoxystrobin was anticipated as both kresoxim-methyl acid (M1) and trifloxystrobin acid (M2) were identified as mentioned above, but detection of azoxystrobin acid was limited in cell culture extracts. To further understand the limited detection of the potential azoxystrobin acid, a follow-up experiment using radiolabeled azoxystrobin was conducted under the experimental conditions identical to nonradiolabeled study as described above. As in the nonradiolabeled study (Figure 2), azoxystrobin was fairly stable in the absence of wheat cells without significant degradation, but was not stable in the presence of the cells, in which only 13.5% of total radioactivity treated was the parent azoxystrobin at 48 HAT (Table 2). The radioactivity other than radiolabel pertaining to azoxystrobin was mainly recovered in aqueous fractions after partitioning against ethyl acetate fractions, and designated as water-soluble unknowns. Previously, metabolism of azoxystrobin to the acid metabolite was reported as a major metabolic route in plants and soil microorganisms.[3,9,10] Thus, azoxystrobin could be demethylated in wheat cell cultures but the demethylated metabolite may not accumulate. It may be possible that the acid metabolite of azoxystrobin, once

Table 2. Percent Recovery of ^{14}C-Azoxystrobin in MS2D Media with (+) or without (−) Wheat Cells at 0, 24, and 48 h after Treatment (HAT)[a]

		Hours after treatment (HAT)		
		0	24	48
− cell	azoxystrobin	98.2 ± 2.7	95.1 ± 2.1	102.9 ± 3.7
	water-soluble unknowns	0.8 ± 0.1	2.0 ± 0.4	1.3 ± 0.2
+ cell	azoxystrobin	96.8 ± 6.0	40.5 ± 2.5	13.5 ± 1.3
	water-soluble unknowns	6.0 ± 0.4	56.4 ± 1.4	75.0 ± 1.3

[a]Azoxystrobin was applied to 7 mL media alone (-) or wheat cell suspension cultures (+; 30 mg/mL) at the rate of 5.0 μg/mL. The total radioactivity of azoxystrobin added to the media or cultures was set to 100%. Data represent means ± standard deviations.

produced, could be rapidly degraded into water-soluble unknowns, limiting the accumulation of the metabolite and subsequently leading to no detection.

In summary, we described the metabolism of three strobilurin fungicides in wheat cell cultures under different experimental conditions. Additionally, we determined the structures of one metabolite of kresoxim-methyl and two metabolites of trifloxystrobin. The findings in this study highlight the differential metabolism of structurally similar strobilurins as well as the dependence of metabolic rates on concentrations of compounds and cells. The findings suggest that the metabolism of strobilurins in plants can be highly diverse. Our results will help us to better understand the metabolic fates of strobilurins in plants.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Ten supplement figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Mike Madary for his technical assistance and Pete Johnson for the preparation of radiolabeled azoxystrobin. We also thank Dow AgroSciences scientists for their helpful suggestions.

■ REFERENCES

(1) Balba, H. Review of strobilurin fungicide chemicals. *J. Environ. Sci. Heal. B* **2007**, *42*, 441−451.

(2) Bartlett, D. W.; Clough, J. M.; Godwin, J. R.; Hall, A. A.; Hamer, M.; Parr-Dobrzanski, B. The strobilurin fungicides. *Pest Manag. Sci.* **2002**, *58*, 649−662.

(3) Roberts, T. R.; Hutson, D. H. *Metabolic Pathways of Agrochemicals Part 2: Insecticides and Fungicides*; The Royal Society of Chemistry: Cambridge, UK, 1999; pp 1329−1342.

(4) European Food Safety Authority. Modification of the existing MRLs for trifloxystrobin in leafy brassica. *EFSA J.* **2010**, *8*, 1648.

(5) Mohapatra, S.; Ahuja, A. K.; Deepa, M.; Jagadish, G. K.; Prakash, G. S.; Kumar, S. Behaviour of trifloxystrobin and tebuconazole on grapes under semi-arid tropical climatic conditions. *Pest Manag. Sci.* **2010**, *66*, 910−915.

(6) Bokern, M.; Nimtz, M.; Harms, H. H. Metabolites of 4-*n*-nonylphenol in wheat cell suspension cultures. *J. Agric. Food Chem.* **1996**, *44*, 1123−1127.

(7) Schmidt, B.; Schuphan, I. Metabolism of the environmental estrogen bisphenol A by plant cell suspension cultures. *Chemosphere* **2002**, *49*, 51−59.

(8) Baloch, R. I.; Yerkes, C. N.; DeBoer, G. J.; Gilbert, J. R. Early stage assessment of metabolic stability in planta. In *Synthesis and Chemistry of Agrochemicals Series VII*; Lyga, J. W., Theodoritis, G., Eds.; Oxford University Press: Cary, NC, 2007; pp 23−36.

(9) Banerjee, K.; Ligon, A. P.; Spiteller, M. Spectral elucidation of the acid metabolites of the four geometric isomers of trifloxystrobin. *Anal. Bioanal. Chem.* **2007**, *388*, 1831−1838.

(10) Clinton, B.; Warden, A. C.; Haboury, S.; Easton, C. J.; Kotsonis, S.; Taylor, M. C.; Oakeshott, J. G.; Russell, R. J.; Scott, C. Bacterial degradation of strobilurin fungicides: a role for a promiscuous methyl esterase activity of the subtilisin proteases? *Biocatal. Biotransform.* **2011**, *29*, 1−11.