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Insights into the Fungicide Prothioconazole and Its Metabolite in Wheat: Residue Behavior and Risk Assessment

Qingkui Fang 1,2,3,†, Zixuan Yan 1,2,†, Chengzhi Zhang 1,2,†, Yanhong Shi 4, Zhaoxian Zhang 4, Quan Gao 1,2, Jinjing Xiao 1,2,3, Min Liao 1,2, Chuanyong Qi 5 and Haiqun Cao 1,2,*

1 Key Laboratory of Agri-Products Quality and Biosafety (Anhui Agricultural University), Ministry of Education, Hefei 230036, China; qkfang@163.com (Q.F.); yzx894852669@163.com (Z.Y.); zcz18451270633@163.com (C.Z.); 15155139755@163.com (Q.G.); xiaojj187012@163.com (J.X.); liaomin3119@126.com (M.L.)
2 Anhui Province Key Laboratory of Integrated Pest Management on Crops, Anhui Agricultural University, Hefei 230036, China
3 Joint Research Center for Food Nutrition and Health of IHM, Hefei 230036, China
4 Provincial Key Laboratory for Agri-Food Safety, College of Resources and Environment, Anhui Agricultural University, Hefei 230036, China; shiyh@ahau.edu.cn (Y.S.); zhangzx@ahau.edu.cn (Z.Z.)
5 Hefei Inspection Center for Agricultural Products Quality, Hefei 230091, China; qichuany@126.com
* Correspondence: haiquncao@163.com
† These authors contributed equally to this work.

Abstract: To clarify the residue formation of prothioconazole and its main metabolite in wheat plants, the uptake, translocation, and metabolism of prothioconazole in wheat roots and leaves were investigated by nutrient solution culture and the spraying method. The results showed that prothioconazole and its metabolites showed a trend of increasing and then decreasing in wheat plants under two treatment methods, and the concentration of prothioconazole and its metabolites was higher in the high-concentration group (5000 µg/mL) than in the low-concentration group (1000 µg/mL). The transferability from stem to leaf was stronger than that from root to stem. In the nutrient solution culture, prothioconazole and its metabolites were mainly enriched in wheat roots. The concentration of prothioconazole in wheat roots increases with the increase in prothioconazole concentration and was significantly higher than the prothioconazole concentration on stems and leaves. In wheat leaves in the spraying method, prothioconazole and its metabolites were conducted from leaves to stems and roots up to the nutrient solution. Prothioconazole-desthio was detectable in wheat nutrient solution, while prothioconazole was not detected. Analysis of actual samples of 9 wheat grains and 28 flours showed that the residues of prothioconazole and its metabolites met the maximum residue limit (0.1 mg/mL) set in China and by the Codex Alimentarius Commission. The results will provide a theoretical basis for the scientific use of prothioconazole and food security assurance.

Keywords: prothioconazole; prothioconazole-desthio; wheat plant; uptake; translocation; metabolism; risk assessment

1. Introduction

Chemical pesticides are important agricultural inputs in agricultural production activities, which not only achieve effective prevention and control of pests, but also help to improve the quality of agricultural products. After chemical pesticides are applied to crops, plants will have the following four endosorption behaviors, including the uptake of pesticides by plant roots, plant stems, plant leaves, fruit, and tubers [1–3], and chemical pesticides form pesticide residues in the plant through endosorption and conduction [4]. Therefore, an in-depth study of the endosorption behavior, dynamic distribution, and accumulation pattern of pesticides in various parts of the plant will help people to clarify the residual behavior of chemical pesticides in the plant, and will also help to explore the mechanism of pesticide control against pests [5,6].
Prothioconazole is a triazole compound whose mechanism is to inhibit the activity of C-14 demethylase in sterol biosynthesis, which in turn disrupts the formation of pathogenic fungal cell membranes, thereby achieving the purpose of being a fungicide [7]. Prothioconazole, developed and created by Bayer, is now sold in more than 60 countries worldwide, including China, and is the third largest product among global fungicides. Prothioconazole has good control effects on wheat diseases such as wheat Fusarium head blight, powdery mildew, sheath blight, etc. Especially wheat Fusarium head blight, which can not only reduce toxins but also increase yield. Therefore, in recent years, prothioconazole has been used increasingly widely. However, unreasonable use may result in the residue of prothioconazole and its metabolites in agricultural products and the environment. The rapid rise of prothioconazole has aroused extensive research interest among scholars, and current research reports on prothioconazole and its metabolites mainly include toxicity assays [8,9], pathogen and toxin prevention and control [10–12], residue analysis method establishment [13], field efficacy [14], residue behavior in environment and crops [15,16], endocrine disruption mechanism [17], etc. Prothioconazole-desthio, the main highly toxic metabolite of prothioconazole, has higher biological activity and endocrine disrupting properties [18,19], and studies have shown that the half-life of prothioconazole-desthio in wheat plants is much longer that of prothioconazole, with longer residual time [13]. Prothioconazole has a wide fungicidal spectrum and a wide range of use, but few studies on the uptake, translocation, and metabolism of prothioconazole in wheat plants have been reported, which is not conducive to the scientific use of prothioconazole, and hinders the further promotion of prothioconazole.

At present, a large number of studies related to the uptake and translocation of pesticides by plants have been reported. For example, Solel et al. [20] studied the leaf absorption of carbendazim in cucumber and apple, and found that carbendazim can be transported bilaterally through phloem. Chandler et al. [21] investigated the absorption of alachlor by wheat and soybean, and found that alachlor was mainly transported through the apoplast pathway, and wheat roots absorbed more alachlor. Bouldin et al. [22] tested the uptake and translocation of atrazine and lambda-cyhalothrin in two aquatic plants by nutrient solution culture. It was found that the uptake and translocation of atrazine and lambda-cyhalothrin reached a maximum at 48 h; atrazine was more easily transported to the upper tissues, while lambda-cyhalothrin was mainly accumulated in the roots. Murano et al. [23] tested the uptake and translocation of diethradin in seven crops, and found that diethradin accumulated mainly in the roots of the crops after absorption. Yamazaki et al. [24] used berberine and perylene to study the different uptake pathways of hydrophilic and hydrophobic compounds in lateral roots of Cucurbita pepo. Li et al. [25] showed that the bound residues of 14C-phenoxymethyl in labeled leaves, labeled fruit pericarp and flesh parts of cucumber plants at maturity showed an increasing trend with time, and the extractable state residues gradually decreased with time. Ju et al. [26] used nutrient solution culture to show that dinotefuran, thiamethoxam, and imidacloprid were absorbed by wheat roots mainly in the cell soluble fractions, which were easily transported and accumulated in the leaves; azoxystrobin, propiconazole and chlorpyrifos were mainly distributed in the cell walls and organelles, so they tend to accumulate in the roots. The above findings provide important theoretical support and technical support for this study. Prothioconazole will be metabolized in plants to produce more toxic prothioconazole-desthio, but the uptake, translocation, and metabolism patterns of prothioconazole in plants are currently unknown, which is not conducive to clarifying the risks of prothioconazole and prothioconazole-desthio, but also not conducive to prothioconazole effectively ensuring the quality and safety of agricultural products.

To clarify the residual behavior of prothioconazole and its main metabolite prothioconazole-desthio in wheat plants, in this study, the fungicide prothioconazole was used as the target, by establishing a residue analysis method for prothioconazole and its metabolite in wheat plant samples. The residue distribution characteristics of prothioconazole and its metabolites in wheat plants were investigated using nutrient solution culture and spraying.
methods, and the residue metabolism pattern of prothioconazole in wheat plants was analyzed. The results will further reveal the uptake, translocation, and metabolism patterns of prothioconazole in wheat plants, and provide some theoretical basis for the scientific use of prothioconazole and food safety assurance.

2. Materials and Methods

2.1. Chemicals and Reagents

The prothioconazole and prothioconazole-desthio standard (purity 99.5%) were from Dr. Ehrenstorfer GmbH (Augsburg, Germany); Octadecylsilyl silane bonded silica gel (C18 50 µm, 60 Å) was purchased from Bonn-Agela Technologies (Tianjin, China); The formic acid (chromatographically pure) was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China); the Acetonitrile (Chromatographical pure), was purchased from the Tedia Company (Fairfield, OH, USA); the 30% prothioconazole suspension was purchased from Anhui Jiuyi Agricultural Co., Ltd. (Hefei, China); the wheat seeds (Sumai 188) were purchased from Jiangsu Fengqing Seed Technology Co., Ltd. (Yangzhou, China).

The autoclave was purchased from Zhiwei Instruments Co., Ltd. (Xiamen, China); the TDL-5A high-speed centrifuge was purchased from Changzhou Yinneng Experimental Instrument Factory (Changzhou, China), the UPF-10 ultrapure water instrument was purchased from Shandong Jingshen Analytical Instruments Co., Ltd. (Zhaozhuang, China); the UPLC-MS/MS, Waters ACQUITY UPLC, Waters Xevo TQD MS (with ESI ion source) were purchased from Waters Technology Co., Ltd. (Shanghai, China); the FA1004N 1/10,000 analytical balance was purchased from Shaoxing Jingmai Instruments & Equipment Co., Ltd. (Shaoxing, China); the VXMTAL multi-tube vortex oscillator was purchased from OHAUS International Trading Co., Ltd. (Shanghai, China); the light incubator was purchased from Shanghai Boxun Medical Biological Instrument Co., Ltd. (Shanghai, China).

2.2. Standard Solutions

Amounts of 2.0 mg (accurate to 0.1 mg) of prothioconazole and prothioconazole-desthio were dissolved in acetonitrile, respectively. Stock solutions of prothioconazole (400 µg/mL) and prothioconazole-desthio (400 µg/mL), for the preparation of prothioconazole and prothioconazole-desthio solutions of different concentration gradients, respectively, were stored in capped glass vials in the dark at −20 °C until required for use.

2.3. Preparation and Cultivation of Wheat Seedlings

The wheat seeds were soaked in 7% (w/w) compound sodium hypochlorite disinfectant solution for 15 min, then the seed surface was rinsed three times with sterile water, and, following this, the seeds were transferred to a glass beaker and soaked in sterile water for 20 h. The soaked seeds were placed on germination trays (325 × 245 × 45 mm) containing sterile moistened germination paper and germinated for 48 h under dark conditions. The germinated wheat seedlings were transferred to plastic square trays (565 × 375 × 80 mm) with sterile nutrient solution (Table S1) and placed in a light incubator to continue incubation for one week. The culture conditions were as follows: the light was turned on from 6:00 a.m. to 22:00 p.m., the light intensity was 250 µmol m−2 s−1, 25/20 °C (light/dark), and relative humidity was maintained at 70% [27].

2.4. Prothioconazole Exposure Experiment

Selecting wheat seedlings of uniform size (root length 11 ± 1 cm, plant height 15 ± 1 cm), the roots were rinsed with sterile water and dried until slightly dry, then transferred to a plastic square tray with nutrient solution. The wheat roots were submerged in the nutrient solution, without the seedling tray and wheat stems touching the nutrient solution. The plastic square plate was wrapped in aluminum foil on all sides, and the gap between the seedling tray and the container was plugged with a sponge wrapped in aluminum foil to prevent photolysis and volatilization of pesticides during culture. Each
processing weighing record was then randomly placed in a light incubator and rerandomized daily. The weight loss of the solution was recorded daily, and the fresh nutrient solution was replenished without the pesticide after aeration.

**Nutrient solution culture method.** We set the prothioconazole concentration in the nutrient solution to 1000 µg/L and 5000 µg/L, and then transfer the prepared wheat plants to a plastic square tray containing prothioconazole nutrient solution. At the same time, the wheat plant group with no prothioconazole in the nutrient solution (blank control) and the wheat-free plant group with prothioconazole in the nutrient solution (remove the loss of prothioconazole when no wheat is absorbed during the experiment) were set as controls. Three replications were set per processing group. Samples of roots, stems, leaves of wheat, and nutrient solution were collected at 0.5, 1, 2, 4, 6, 10, 24, 48, 96, 144, 192, 240, 288, and 336 h. Since wheat roots were soaked in a higher concentration of prothioconazole-containing nutrient solution, the root were rinsed with acetonitrile during sampling (10 mL × 3 times × 10 s), and then rinsed with tap water (200 mL × 1 time × 30 s), and then 10 mL of acetonitrile (10 mL × 1 time × 10 s) so as to remove the pesticide residues on the root surface. The collected root, stem and leaf of wheat samples were stored at −20 °C, the nutrient solution was stored at −4 °C, and kept in dark place.

**Spraying method.** According to the recommended dose and 1.5 times the recommended dose of 30% prothioconazole suspension in wheat fields, we set up two groups of treatment concentration, and diluted the prothioconazole suspension with sterile water at an amount of 45 mL/m². We moved the prepared wheat plants to a plastic square tray with nutrient solution and sprayed the prepared prothioconazole suspension on the leaves, avoiding spraying to the roots. After spraying, we rinsed the wheat roots with sterile water (200 mL × 3 times × 10 s) and replaced the nutrient solution to ensure that there is no prothioconazole residue in the nutrient solution. At the same time, the water spray group was set as a control. Three replications were set per processing group. Samples of roots, stems, leaves of wheat, and nutrient solution were collected at 0.25, 0.5, 1, 2, 4, 8, 10, 12, 24, 48, 96, 144, 192, 240, 288, and 336 h. Since prothioconazole suspension is sprayed directly on the leaves of wheat, the leaf surface is first rinsed with acetonitrile (10 mL × 3 times × 10 s), then rinsed with tap water (200 mL × 1 time × 30 s), and then rinsed with acetonitrile (10 mL × 1 time × 10 s) to remove residual pesticides on the wheat leaf surface. Unwashed wheat leaves were also collected to measure the amount of prothioconazole deposition on the wheat leaves. The root, stem, and wheat leaf samples collected each time were stored at −20 °C, the nutrient solution was stored at −4 °C, and kept in dark place.

2.5. Wheat Grain and Flour Sample Collection

The distribution of wheat grain and wheat flour sample collection areas is shown in (Table 1). Wheat grain samples were collected from 10 provinces and cities in China, each sample was not less than 0.5 kg, and was stored at 4 °C. Wheat flour samples were collected in 28 counties and cities in Anhui Province, and the purchased wheat flour was local bulk flour, each sample was not less than 0.5 kg, and was stored at 4 °C.

2.6. Sample Preparation

Wheat plant samples were collected for each of the treatment experiments. The roots, stems, and leaves of wheat plants were triturated separately under the condition of liquid nitrogen, and homogenized for analysis.
Table 1. Test results of wheat grain and wheat flour market samples.

<table>
<thead>
<tr>
<th>Wheat Grain</th>
<th>Wheat Flour</th>
<th>Wheat Flour</th>
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<tbody>
<tr>
<td>Num</td>
<td>Prothioconazole (µg/kg)</td>
<td>Prothioconazole-Desthio (µg/kg)</td>
</tr>
<tr>
<td>1</td>
<td>&lt;LOD a</td>
<td>&lt;LOD</td>
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<td>2</td>
<td>&lt;LOD</td>
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<tr>
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<td>&lt;LOD</td>
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<tr>
<td>8</td>
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<td>&lt;LOD</td>
</tr>
<tr>
<td>9</td>
<td>64.3</td>
<td>6.8</td>
</tr>
</tbody>
</table>

a: <limited of detection (LOD).
2.7. Sample Extraction

**Wheat plant samples** One gram of homogenized root, stem, and leaf of wheat sample was added to a 50 mL centrifuge tube, and then 10 mL of acetonitrile (4 °C pre-cooling) was added, vortexed for 2 min, followed by adding one gram of NaCl and one gram of anhydrous MgSO₄, vortexed for 2 min, then centrifuged at 5000 r/min for 5 min (4 °C), and 2 mL of supernatant was taken to be purified.

**Wheat grain and flour samples** Two grams of wheat grain sample (after homogenization) and flour sample were added to a 50 mL centrifuge tube, and then 10 mL of acetonitrile (4 °C pre-cooling) was added, vortexed for 2 min, followed by adding one gram NaCl, vortexed for 2 min, then centrifuged at 5000 r/min for 5 min (4 °C), and 2 mL of supernatant was taken to be purified.

**Nutrient solution sample** One milliliter of nutrient solution was added to a 50 mL centrifuge tube. And then 10 mL of acetonitrile (4 °C pre-cooling) was added, vortexed for 2 min, followed by adding one gram of NaCl and one gram of anhydrous MgSO₄, vortexed for another 2 min, then centrifuged at 5000 r/min for 5 min (4 °C), one milliliter of supernatant was taken and passed through the 0.22 µm organic filter membrane to be detected.

2.8. Clean-Up

The extraction supernatant of wheat root, grain, and flour samples was taken (pre-cooled at 4 °C), and 100 mg anhydrous MgSO₄ and 50 mg C18 were added to a 15 mL centrifuge tube. The mixture was vortexed for 2 min and then centrifuged at 5000 r/min for 5 min (at 4 °C). One milliliter of supernatant was taken and passed through a 0.22 µm organic filter membrane for detection.

The extraction supernatant of wheat stem and leaf samples was taken, and 100 mg of anhydrous MgSO₄, 50 mg of C18, and 20 mg of GCB were added to a 15 mL centrifuge tube. The mixture was vortexed for 2 min and then centrifuged at 5000 r/min for 5 min. One milliliter of supernatant was taken and passed through a 0.22 µm organic filter for detection.

2.9. Evaluation of Matrix Effects

To accurately evaluate the influence of each matrix on the accuracy of the assay results, and prepare concentration gradients of solvent mixed standard solution and matrix mixed standard solution separately, standard curves were plotted, and the matrix effect (ME) was calculated. The formula for calculating the matrix effect is ME(%) = \( \left( \frac{S_1}{S_0} - 1 \right) \times 100% \). Where \( S_0 \) is the slope of the linear equation of the solvent-mixed standard solution; \( S_1 \) is the slope of the linear equation of the matrix-mixed standard working solution. When |ME| < 20%, the matrix effect can be neglected; 20% < |ME| < 50% indicates the presence of moderate matrix effect; |ME| > 50% indicates the presence of strong matrix effect [28].

2.10. Detection Testing Conditions

The LC-MS/MS methods were prepared as described previously [13], with slight modification. An Acquity UPLC equipped with a BEH (ethylene bridged hybrid) C18 column (50 mm × 2.1 mm, 1.7 µm particle size; Waters, Milford, MA, USA) was coupled to a Xevo TQ triple-quadrupole-mass spectrometer (Waters) and operated in the positive electrospray ionization mode for UPLC-MS/MS. The LC was operated under gradient conditions with mobile phases of A (water + 0.1% formic acid) and B (acetonitrile) at 40 °C, and A:B = 20:80 (v/v). The mobile phase flow rate was 0.4 mL/min. The total run time was 3.5 min. The sample injection volume was 10.0 µL.

The mass spectrometry source temperature was 150 °C, and the nitrogen gas flow rates for the cone and desolvation gases were 150.0 and 1000.0 L/h, respectively. The desolvation temperature was 500 °C. Argon was used as the collision gas with a flow rate of 3.0 mL/min. The mass spectrometer was operated in the multiple reactions monitoring mode, and two precursor/product ion transitions were monitored for each analyte. The quantification and confirmation calculations were performed using the software Target Lynx 4.1 (Waters Corp.,
Milford, MA, USA). Ion transitions, cone voltages, collision energies, and dwell times for the analytes are shown in Table S2.

2.11. Data Statistics and Analysis

In order to evaluate the bioaccumulation capacity of prothioconazole in wheat plants, this study used bioconcentration factors (BCFs) to assess the enrichment capacity of wheat plant roots, stems, and leaves for the uptake of prothioconazole in nutrient solution [29,30]. Including root bioconcentration factor (RCF), stem bioconcentration factor (SCF) and leaf bioconcentration factor (LCF), the calculation formula is RCF = \( C_{\text{root}} / C_{\text{water}} \), SCF = \( C_{\text{stem}} / C_{\text{water}} \) and LCF = \( C_{\text{leaf}} / C_{\text{water}} \).

In order to evaluate the transport capacity of prothioconazole in wheat roots, stems, and leaves, the degree of pesticide transport in wheat plants was expressed by transport factors (translocation factors, TFs), root-to-stem (TF\(_{\text{stem/root}}\)) and stem-to-leaf (TF\(_{\text{leaf/stem}}\)) [31], the calculation formula is TF\(_{\text{stem/root}}\) = \( C_{\text{stem}} / C_{\text{root}} \) and TF\(_{\text{leaf/stem}}\) = \( C_{\text{leaf}} / C_{\text{stem}} \). Where \( C_{\text{water}} \) represents the concentration of pesticides in the nutrient solution; \( C_{\text{root}} \) represents the concentration of pesticides in wheat roots; \( C_{\text{stem}} \) represents pesticide concentration in wheat stems; \( C_{\text{leaf}} \) represents the concentration of pesticides in wheat leaves. The digestion of pesticides in nutrient solution is fitted using the first-order kinetic equation [32]: The equation is \( C_t = C_0 \times e^{-kt} \). Where \( C_t \) represents the concentration of time in the nutrient solution at t time (mg/L); \( C_0 \) represents the initial concentration in the nutrient solution; \( k \) represents the first-order digestion constant. Among them, the half-life is calculated as follows: \( T1/2 = \ln2 / k = 0.639 / k \).

3. Results and Discussion

3.1. Reliability of Analytical Methods for Prothioconazole and Prothioconazole-Desthio in Wheat Substrates and Nutrient Solutions

The linear relationship between prothioconazole concentration and peak area was good in the concentration range of 2~400 µg/L with a correlation coefficient \( (R^2) \) of 0.9999. The limit of quantification (LOQ) of prothioconazole was 2 µg/L (10 × S/N ratio) and the limit of detection (LOD) was 0.7 µg/kg (3 × S/N ratio) [33]. The linear relationship between the concentration and peak area of prothioconazole-desthio was good in the concentration range of 2~50 µg/L with a correlation coefficient \( (R^2) \) of 1. The LOQ of prothioconazole-desthio was 0.2 µg/L (10 × S/N ratio) and the LOD was 0.08 µg/kg (3 × S/N ratio).

The recovery results of prothioconazole and prothioconazole-desthio, which spiked in six matrices of wheat roots, stems, leaves, nutrient solution, grain, and flour, are shown in Table S3. The concentration of prothioconazole in the six matrices ranged from 50 to ~400 µg/kg, the recovery rate was 75.1~112.6%, the relative standard deviations were 0.4~9.9%, and the matrix effect was 3.1~23.5%. The concentration of prothioconazole-desthio in the six matrices ranged from 2 to ~100 µg/kg, the recovery rate was 82.3~117.0%, the relative standard deviations were 0.2~7.8%, and the matrix effect was 2.5~20.1%. The extraction and purification of prothioconazole at room temperature are very easy to degrade. In this study, the recovery tests were well carried out by keeping the low temperature (4 °C) during operation. From the results, the method is suitable for the residue analysis of prothioconazole and its metabolites in wheat plants and nutrient solution.

3.2. Uptake, Transport, and Metabolic Patterns of Prothioconazole in Wheat Plants by Nutrient Solution Culture

In the nutrient solution culture method, the prothioconazole added to the nutrient solution is taken up by the roots of wheat plants, and is transported and metabolized upward in the stem and leaf of wheat plants. The concentration of prothioconazole and its metabolites in the roots, stems, and leaves of wheat changes with time as shown in Figure 1 (Figure 1A, low-concentration group: 1000 µg/kg; Figure 1B, high-concentration group: 5000 µg/kg). In wheat plants, the concentration of prothioconazole and its metabolite prothioconazole-desthio showed a trend of increasing and then decreasing. The concentra-
tion of prothioconazole and its metabolite was higher in the high-concentration group than in the low-concentration group.

![Absorption and metabolism results of prothioconazole and prothioconazole-desthio in wheat roots, stems, and leaves](image)

**Figure 1.** Absorption and metabolism results of prothioconazole and prothioconazole-desthio in wheat roots, stems, and leaves ((A): 1000 µg/L, (B): 5000 µg/L).

In wheat roots, prothioconazole in the low-concentration group gradually increased and peaked at 642.5 µg/kg at 20 h, and then the prothioconazole gradually decreased. In the high-concentration group, prothioconazole rapidly increased and peaked at 1574.6 µg/kg at 10 h. In wheat stems and leaves, prothioconazole concentrations peaked from 0–10 h to 24 h. Prothioconazole concentrations were 433.9 µg/kg in stems and 418.1 µg/kg in leaves for the low-concentration group, and 779.8 µg/kg in stems and 573.5 µg/kg in leaves for the high-concentration group. The results indicate that within a certain concentration range, the uptake of prothioconazole by wheat roots increases with the increase of pesticide concentration, and the distribution of pesticide residue level varies in different plant tissues.

Prothioconazole-desthio is the main metabolite of prothioconazole, prothioconazole is rapidly photolyzed into the metabolite prothioconazole-desthio in water and plants. In wheat roots, the concentration of prothioconazole-desthio in the low-concentration prothioconazole group increased rapidly at 24 h and reached a peak concentration of 1544.9 µg/kg. The concentration of prothioconazole-desthio in the high-concentration prothioconazole group increased rapidly and reached a peak of 2718.5 µg/kg at 4 h. In the
stem and leaves of wheat, the concentration of prothioconazole-dethio peaked at 10 h and 144 h. In the low-concentration group, the concentration of prothioconazole-dethio in the stem was 227.7 µg/kg and 536.1 µg/kg in the leaves; in the high-concentration group, the concentration of prothioconazole-dethio in the stem was 858.0 µg/kg and 678.8 µg/kg in the leaves. The results showed that after the application of prothioconazole, the degradation rate was fast, and the residual concentration of the metabolite prothioconazole-dethio was high. Considering the higher toxicity of the metabolite, the detection of prothioconazole in agricultural products should pay attention to the residual level of prothioconazole-dethio.

Miller et al. [34] reported that compounds with low solubility in water and partition coefficient in octanol-water >1.8 are more likely to accumulate in plant roots and are not easy to transport upward. Trapp [35] studies the weak acid compounds with medium hydrophobicity and found they are transported in the phloem of plants, so the compounds will be transported up and down at the same time, resulting in less accumulation in leaves. The solubility of prothioconazole in water was low at 0.3 g/L, the logarithmic value of the partition coefficient of prothioconazole in octanol-water is 4.05, and the acidity coefficient of prothioconazole at room temperature is 6.9. The experimental results showed that the upward transport of prothioconazole in wheat plants was not strong and it was easily adsorbed by the roots. At the same time, the concentration of prothioconazole in the roots of wheat plants was higher than that in the stems and leaves by nutrient solution culture, and the trend was more significant in the high-concentration group, resulting in a significantly higher concentration of prothioconazole-dethio in the roots of wheat plants than that in the parent prothioconazole.

3.3. Bioconcentration and Transport of Prothioconazole in Wheat Plants by Nutrient Solution Culture

There were differences in the bioconcentration capacity of different parts of wheat plants for prothioconazole, and the bioconcentration results are shown in Figure 2A,B. In the low-concentration group, the 0–10 h root bioconcentration factor (RCF) values increased with time; the 10 h–336 h RCF values stabilized, RCF value was more than 1.10 L/kg, indicating that the wheat roots reached an equilibrium state for the uptake of prothioconazole in the nutrient solution. In the high-concentration group, the RCF value increased with time from 0 to 192 h and reached a peak value of 1.60 L/kg. The mean RCF values were 1.60 L/kg and 1.07 L/kg for the low- and high-concentration groups, respectively, and the mean RCF values were higher for the low-concentration group than the high-concentration group. The stem bioconcentration factor (SCF) and leaf bioconcentration factor (LCF) of wheat gradually increased from 0 to 24 h and slightly decreased before increasing at 24 h–336 h. The mean values of SCF were 1.21 L/kg and 0.18 L/kg in the low-concentration group and the high-concentration group, respectively. The mean values of LCF were 0.95 L/kg and 0.20 L/kg in the low-concentration group and the high-concentration group, respectively. The enrichment factors of wheat roots, stems, and leaves showed an increasing trend after 288 h, probably due to the disintegration of prothioconazole in wheat plants, resulting in changes in bioconcentration values. Based on the experimental results, the RCF of wheat plants was higher than the SCF and LCF. The bioconcentration capacity of prothioconazole in wheat roots was higher than that in stems and leaves, and the mean values of RCF, SCF and LCF in the low-concentration group were significantly higher than those in the high-concentration group, indicating that the bioconcentration capacity of wheat plants was affected by the increase in prothioconazole concentration.

The concentration of prothioconazole had an effect on the translocation capacity of wheat plants, and the results of translocation factors (TFs) values of prothioconazole in wheat plants over time are shown in (Figure 2C,D). When the prothioconazole in nutrient solution was 1000 µg/L, TFleaf/stem in wheat plants was slightly higher than TFstem/root, indicating that prothioconazole was slightly easier to translocate from stems to leaves than from roots to stems in the low-concentration group. However, the TFstem/root and TFleaf/stem values were both around 1, indicating that wheat plants did not have strong
translocation from roots to stems and from stems to leaves at a low-concentration of prothioconazole. When the concentration of prothioconazole in the nutrient solution was 5000 µg/L, the TF_{leaf/stem} values ranged from 0.6 to 3.7, and the translocation capacity from stem to leaf was enhanced; the TF_{stem/root} values ranged from 0.2 to 0.6, indicating that the root uptake level was increased with the increase of prothioconazole concentration, while the translocation capacity to the stem did not improve.

Dodgen et al. [36] showed that when pesticides enter the plant stem, the transport from stem to leaf is influenced by transpiration intensity as well as the pesticide's own physico-chemical properties. The results of Ju’s study showed that the different physicochemical properties of the pesticides themselves caused the differences in pesticide translocation capacity and demonstrated that the translocation capacity of nonionic pesticides from roots to stems to leaves was decreasing due to the increase in hydrophobicity and decrease in water solubility [10]. Prothioconazole has higher hydrophobicity and lower water solubility, so the transport capacity of prothioconazole in wheat plants is weak. The results of the above study are consistent with the results of this experiment.

3.4. Uptake and Transport of Prothioconazole in Wheat under Replacement of Nutrient Solution

Under the condition of changing the nutrient solution containing prothioconazole once in 48 h, the concentration of prothioconazole in wheat roots and leaves with time is shown in Figure 3. In the low-concentration group, the concentration of prothioconazole in wheat roots continued to increase from 0 to 432 h, peaking at 1656.5 µg/L. In the high-concentration group, the concentration of prothioconazole in wheat roots gradually increased from 0 to 240 h, peaking at 3959.7 µg/L. The decreasing trend of prothioconazole concentration was observed in the later stages of both treatment groups, probably because the wheat plants started to age and their metabolic capacity was weakened. Combined with the results in Figure 1, it showed that the bioconcentration of prothioconazole in wheat roots gradually increased with the increase in prothioconazole concentration in the nutrient solution and was significantly higher than the prothioconazole concentration in stems and leaves. Also, the prothioconazole concentrations in wheat stems and leaves were slightly higher than those in the treatment group with one application. The results further revealed that prothioconazole was easily bioconcentrated in wheat roots and not
easily transported to wheat stems and leaves in wheat plants, and excessive or repeated application of pesticides may cause a large accumulation of prothioconazole in the plant.

![Graph showing bioconcentration and transport results of prothioconazole](image)

**Figure 2.** Bioconcentration and transport results of prothioconazole in wheat roots, stems, and leaves with time ((A): 1000 µg/L, (B): 5000 µg/L).

### 3.5. Residual Dynamics of Prothioconazole in Nutrient Solution Culture

The residual dissipation dynamics of prothioconazole in the nutrient solution is shown in Figure 3. In the plant-free treatment group of prothioconazole, prothioconazole was first in a stable state and then gradually decreased. The residues of prothioconazole in the low and high concentrations dissipated by 93.0% and 78.7% after 14 d. The half-lives of prothioconazole in the control group were 3.6 d and 5.7 d, respectively. In the treatment group, prothioconazole showed a gradually decreasing trend, and the residues of prothioconazole in the low- and high-concentration groups dissipated by 98.7% and 94.9% after 14 d. The half-lives of prothioconazole in the control group were 1.06 d and 2.63 d, respectively (Table S4). The experimental results showed that the dissipation of the high concentrations of prothioconazole in the nutrient solution was a gradually decreasing process, and the dissipation of the low-concentration of prothioconazole in the nutrient solution was faster than that of the high-concentration group. Among other things, the faster initial concentration decrease in the low-concentration group might be due to the rapid uptake of prothioconazole by wheat roots, while in the high-concentration group this had limited effect due to weaker uptake.

![Graph showing dissipation curves of prothioconazole](image)

**Figure 3.** Variation in prothioconazole concentration in wheat roots, stems, and leaves with time ((A): 1000 µg/L, (B): 5000 µg/L).

### 3.6. Uptake, Transport, and Metabolism of Prothioconazole in Wheat Plants by Spraying Method

In the spraying method, prothioconazole on the leaf surface is absorbed by the leaves of wheat plants and is transported and metabolized in the stems and roots, and the concentration of prothioconazole and its metabolites in wheat roots, stems, and leaves over
time is shown in Figure 5. In wheat plants, the concentration of prothioconazole and its metabolite prothioconazole-desthio showed a trend of increasing and then decreasing, and the concentration of prothioconazole and its metabolites in the high-concentration group was higher than that in the low-concentration group.

![Figure 5](image)

**Figure 5.** Changes in the concentration of prothioconazole and its metabolites in wheat roots, stems, leaves and nutrient solution with time. (A), Leaves (1000 µg/L); (B), leaves (5000 µg/L); (C), stems (1000 µg/L); (D), stems (5000 µg/L); (E), roots (1000 µg/L); (F), roots (5000 µg/L); (G), nutrient solution (1000 µg/L); (H), nutrient solution (5000 µg/L).

**Wheat leaves.** The uptake and metabolism of prothioconazole on the leaf surface by wheat plants are shown in Figure 5A,B. Prothioconazole in leaves gradually rose to a peak concentration of 116.5 µg/kg from 0 to 24 h. In the low and high-concentration groups, the main metabolite prothioconazole-desthio rose rapidly from 0 to 8 h and reached a peak concentration of 225.9 µg/kg and 652.5 µg/kg, respectively. The concentration of prothioconazole-desthio in the leaves was higher than that of the parent prothioconazole, probably due to the rapid dissipation of prothioconazole to prothioconazole-desthio on the leaf surface by light, which was absorbed by the leaf tissue cells and stomata.

**Wheat stems.** Prothioconazole in wheat stems was mainly transported by leaves, as well as stem to stem surface during prothioconazole uptake. In the low-concentration group (Figure 5C), the prothioconazole in wheat stems gradually increased to a peak concentration of 98.3 µg/kg at 0–96 h. In the high-concentration group (Figure 5D), prothioconazole in wheat stems gradually increased to a peak concentration of 138.6 µg/kg at 12 h. In the low- and high-concentration groups, the main metabolite prothioconazole-desthio increased rapidly from 0 to 12 h and 0 to 48 h with concentrations of 101.7 µg/kg and 278.3 µg/kg, respectively; from 12 h to 336 h, prothioconazole-desthio decreased gradually and then increased slightly, which may be related to the bidirectional conduction and dissipation of prothioconazole in the wheat stems. Deletage-Grandon’s study showed that during the bidirectional conduction of compounds in the plant, there is a constant diffusion of molecules into the plastid extracellular body moving with the transpiration flow [37].

**Wheat roots.** The prothioconazole absorbed by wheat leaves could be transmitted down to the roots. The change rule of prothioconazole in wheat roots is shown in Figure 5E,F. In the low-concentration group, prothioconazole in wheat roots gradually rose to a peak concentration of 94.7 µg/kg at 0–24 h. In the high-concentration group, prothioconazole in wheat roots gradually rose to a peak concentration of 140.7 µg/kg at 0–48 h. In the low- and high-concentration groups, the main metabolite prothioconazole-desthio increased rapidly from 0 to 24 h with concentrations of 120.7 µg/kg and 123.5 µg/kg, respectively. The concentration of prothioconazole in the roots was lower than that in the stems and leaves of wheat, indicating that prothioconazole was not easily transmitted.
downward from the leaves of wheat. Compared to the nutrient solution culture method, the spraying method has lower residual amounts of prothioconazole and metabolites in the roots and stems of the plants. Therefore, especially for pesticides that are not easily transmitted downwards, spraying methods may be more conducive to ensuring the safety of root and stem agricultural products when controlling plant leaf diseases and pests.

**Nutrient solution.** The results of prothioconazole and its metabolites in the nutrient solution are shown in Figure 5G,H. From the results, prothioconazole was not detected in the nutrient solution, indicating that prothioconazole in the wheat roots could not be transferred to the nutrient solution or a small part of it was transferred and decomposed rapidly. Prothioconazole-desthi only in wheat roots could be transferred to the nutrient solution, and the average concentration of prothioconazole-desthi in the low-concentration group was at 18.9 µg/kg. The concentration of prothioconazole-desthi in the high-concentration group increased and then decreased with a peak concentration of 62.7 µg/kg.

In the conventional studies of pesticide residue behavior, two-three sampling time points are usually selected to observe the change in pesticide residue within 0–24 h of exposure [32]. And in this study, by setting seven-eight sampling time points at 0–24 h of pesticide exposure, a trend of increasing and then decreasing concentration of prothioconazole and its metabolite prothioconazole-desthi was observed. In the nutrient solution culture and spray method, the residual peak of prothioconazole and prothioconazole-desthi appeared in about 10 h many times. Therefore, by optimizing the sampling time within 24 h, an effective scheme is provided for studying the absorption, transport and metabolism of prothioconazole and its metabolites in wheat plant.

### 3.7. Dissipation of the Prothioconazole in Wheat Leaves of the Spraying Method

The data on residue dynamics obtained for prothioconazole in the wheat leaves are shown in Table 2. A gradual and continuous deterioration of pesticide residues in the two treated samples was observed. The average residues of prothioconazole in the leaves were 704.1 µg/kg and 1794.6 µg/kg, respectively, after 2 h of the application, and the residue level of the two pesticides dissipated by 95.9% and 93.6%, respectively, after 12 days. The half-lives of prothioconazole in the leaves were 3.2 and 3.6 d, respectively, and the dynamics are described by the equations $C = 0.3721e^{-0.009t}$, $R^2 = 0.893$ and $C = 1.0582e^{-0.008t}$, $R^2 = 0.918$, respectively. The prothioconazole dissipated faster in leaves; on the basis of previously published studies [38,39], sunlight, evaporation, and rainfall elution are important factors affecting the dissipation of pesticides, especially sunlight photodegradation.

#### Table 2. Results of dissipation of prothioconazole in wheat leaves.

<table>
<thead>
<tr>
<th>Time (d)</th>
<th>Low-Concentration Group</th>
<th>High-Concentration Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Residue Level (µg/kg)</td>
<td>Dissipation Rate (%)</td>
</tr>
<tr>
<td>0</td>
<td>704.1</td>
<td>— —</td>
</tr>
<tr>
<td>1</td>
<td>234.3</td>
<td>66.7</td>
</tr>
<tr>
<td>2</td>
<td>187.3</td>
<td>73.4</td>
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<td>4</td>
<td>121.0</td>
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<tr>
<td>6</td>
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<td>86.5</td>
</tr>
<tr>
<td>8</td>
<td>68.5</td>
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</tr>
<tr>
<td>10</td>
<td>59.8</td>
<td>91.5</td>
</tr>
<tr>
<td>12</td>
<td>28.7</td>
<td>95.9</td>
</tr>
</tbody>
</table>

Dissolution equation $C = 0.3721e^{-0.009t}$
Correlation coefficient $R^2 = 0.893$
Half-life (d) $t_{1/2} = 3.2$

Dissolution equation $C = 1.0582e^{-0.008t}$
Correlation coefficient $R^2 = 0.918$
Half-life (d) $t_{1/2} = 3.6$
3.8. Residue Analysis of Prothioconazole and Its Metabolites in Wheat Grain and Flour Market Samples

To prevent and control diseases in wheat cultivation, producers may use prothioconazole, resulting in direct residues and absorbed enriched residues of prothioconazole and its metabolites on the grain. In order to assess the possible risks, we analyzed the residues of prothioconazole in wheat grains and flours from market samples. Authentic wheat grain and flour samples were collected from retail stores and supermarkets in China. The concentrations of the residues of the prothioconazole and prothioconazole-desthio in these samples were detected (Table 2). In the nine wheat grain samples and the 28 flour samples, the concentration ranges of the residues were: LOD–86.3 µg/kg for prothioconazole and LOD–12.5 µg/kg for prothioconazole-desthio in the wheat grain; LOD–18.8 µg/kg for prothioconazole and LOD–13.1 µg/kg for prothioconazole-desthio in the flour. The JMPR (Joint Meeting on Pesticide Residues) pointed out that the prothioconazole residue definition for risk assessment in plant commodities is the metabolite prothioconazole-desthio [40]. According to these results, the milling treatment of wheat grain reduced the residues of prothioconazole, probably due to the partial distribution of residues into the bran after processing. This is consistent with the results of the toxin distribution of wheat grain after processing [41] and the distribution of pesticide residues in pears [42] and apples [43] after peeling. Based on the results, the concentrations of prothioconazole and prothioconazole-desthio residues in the wheat grain and flour did not exceed the maximum residue limit (0.1 mg/kg) in the Codex Alimentarius Commission [44] and in China. Currently, the maximum residue limits for pesticides in food are mainly based on the residues in primary agricultural products. According to the research results of this article, preliminary processing such as wheat milling can to some extent affect the pesticide residues in agricultural products. Therefore, directly evaluating pesticide residues in primary agricultural products may overestimate or underestimate the risk of pesticide residues.

4. Conclusions

In this study, the uptake, translocation, and metabolism of the fungicide prothioconazole and its major metabolite in wheat plant were investigated by nutrient solution culture and spraying method. Under two treatment methods, prothioconazole and its metabolites showed a trend of increasing and then decreasing in wheat plants. In the nutrient solution culture, prothioconazole and its metabolites were mainly enriched in wheat roots. The transferability from stem to leaf was stronger than that from root to stem. In wheat leaves in the spraying method, prothioconazole in leaves gradually rose to a peak concentration at 24 h. In the low- and high-concentration groups, the main metabolite prothioconazole-desthio rose rapidly and reached a peak concentration. By optimizing the number of sampling analysis within 24 h of pesticide exposure, a trend of increasing and then decreasing concentration of prothioconazole and its metabolite was observed. Analysis of 37 authentic samples showed the residue concentrations of prothioconazole and prothioconazole-desthio met the requirements of the China and the Codex Alimentarius Commission.

The uptake, translocation, and metabolism of pesticides in plants and the environment are comprehensively influenced by various factors. This article mainly studies three variables: pesticide application method, time, and pesticide concentration. And of course, light and temperature are also important factors. Light may not have a significant impact on pesticide metabolism inside the plant, but it can affect pesticide metabolism on the surface of the plant. Within a certain range, the stronger the light intensity, the faster the pesticide degrades. High or low temperatures can affect the uptake, translocation, and metabolism of pesticides, possibly affecting their volatilization, biodegradation, and chemical degradation pathways [45,46].
**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13122906/s1. Table S1. Composition of the nutrient solution. Table S2. MRM detection conditions for UPLC-MS/MS analysis of prothioconazole and its metabolites. Table S3. Recovery, coefficient of variation and matrix standard curves of prothioconazole and prothioconazole-dethio spiked in six matrices. Table S4. Primary kinetic parameters of prothioconazole in nutrient solution.

**Author Contributions:** Conceptualization, Q.F., J.X., M.L. and H.C.; methodology, Q.F., C.Z. and Z.Z.; software, C.Z.; validation, Z.Y., C.Z. and Q.G.; investigation, Z.Y., C.Z., Z.Z. and Q.G.; resources, Q.F.; data curation, Q.F., C.Z., J.X. and C.Q.; writing—original draft, Q.F., Z.Y. and C.Z.; writing—review and editing, Q.F., Z.Y., Y.S. and Z.Z.; supervision, Q.F., Y.S., M.L., C.Q. and H.C.; funding acquisition, Q.F. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was supported by the Natural Science Key Research Project of Colleges and Universities in Anhui Province (KJ2020ZD11), the Research Funds of Joint Research Center for Food Nutrition and Health of IHM (2023SY01), and the University Synergy Innovation Program of Anhui Province (GXXT-2021-059).

**Data Availability Statement:** The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

**Conflicts of Interest:** The authors declare no conflict of interest.

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