

## Article

# Field-Evolved Sulfoxaflor Resistance of Three Wheat Aphid Species in China

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**Abstract:** Sulfoxaflor belongs to a new class of insecticides which are effective against many sap-feeding pests. *Sitobion miscanthi*, *Rhopalosiphum padi*, and *Metopolophium dirhodum* are the predominant pests coexisting on wheat plants. It is unknown whether these aphid species have developed resistance to sulfoxaflor. Here, the susceptibilities of three wheat aphid species from different regions of China to sulfoxaflor were evaluated. The results showed that two *S. miscanthi*, one *R. padi*, and two *M. dirhodum* field populations were highly resistant to sulfoxaflor. Additionally, 13 *S. miscanthi*, 9 *R. padi*, and 4 *M. dirhodum* field populations were moderately resistant to sulfoxaflor. Analysis of differences in toxicity showed that the susceptibility levels of *R. padi* in 9 of 20 regions, *M. dirhodum* in 5 of 9 regions, and *M. dirhodum* in 3 of 9 regions to sulfoxaflor were greater than those of *S. miscanthi*, *S. miscanthi*, and *R. padi* in the same regions, respectively. Thus, each wheat aphid species has field populations that are highly sulfoxaflor resistant. The *R. padi* and *M. dirhodum* populations were more susceptible to sulfoxaflor than those of *S. miscanthi*. These findings provide new insights into insecticide resistance development and rational sulfoxaflor use.

**Keywords:** insecticide resistance; sulfoxaflor; toxicity difference; wheat aphid



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## 1. Introduction

Sulfoxaflor, which belongs to a new class of insecticides (sulfoximines), is highly effective in controlling many kinds of sap-feeding pests [1–3]. Sulfoximines are unique among commercial insecticides in that they contain sulfoximine functional groups [2]. The target sites of sulfoxaflor are nicotinic acetylcholine receptors in the insect nervous system. The poisoned insects show abnormal excitement levels and then become paralyzed, resulting in death [1]. However, the chemical and biochemical properties of sulfoxaflor differ from those of other insecticides that target nicotinic acetylcholine receptors, including spinosyns, neonicotinoids, and nereistoxin analogs [3]. Therefore, sulfoxaflor has been selected for commercial development and may be useful for controlling many pests that have developed resistance to neonicotinoids and other insecticides [2,4–6]. In 2014, sulfoxaflor was registered in China as an important new option for controlling wheat aphids. To the best of our knowledge, this novel insecticide has not been used continuously over a prolonged period to control wheat aphids in most areas of China [7]. Furthermore, there are no reports of sulfoxaflor resistance in wheat aphids.

*Sitobion miscanthi*, *Rhopalosiphum padi* (Linnaeus), and *Metopolophium dirhodum* (Walker) are the major aphids (Hemiptera: Aphididae) that infest cereal crops. Both *S. miscanthi* and *R. padi* have been detected in all wheat fields in China [8,9]. In contrast, *M. dirhodum* is mainly distributed at high altitudes and in the northwestern regions of China [7]. These three wheat aphid species usually coexist on growing wheat plants. They can severely damage crops by feeding on plants or by acting as vectors for a variety of plant pathogenic viruses [10]. Global crop losses due to aphid infestations are estimated to be in the hundreds of millions of dollars per year [11]. In China, 10–15 million hectares are infested with cereal aphids, resulting in 10% yield losses annually [9,12,13]. In 2020, the National Agro-Tech Extension and Service Center of China released a report describing a 55.9% year-by-year increase in wheat aphid infestations.

Previous monitoring results revealed that most wheat aphid populations are still sensitive or exhibit low but gradually increasing resistance to various insecticides [14,15]. Recent studies confirmed that *S. miscanthi*, *R. padi*, and *M. dirhodum* field populations have developed resistance to many insecticides, including neonicotinoids (thiamethoxam and imidacloprid), pyrethroids (bifenthrin and beta-cypermethrin), macrolides (avermectin), and organophosphates (chlorpyrifos and omethoate) [7,8]. Regarding sulfoxaflor resistance, various *Nilaparvata lugens* and *Aphis gossypii* field populations with low resistance levels have been identified [16,17].

It is unknown whether wheat aphids have developed resistance to sulfoxaflor. Here, we evaluated the susceptibility of *S. miscanthi*, *R. padi*, and *M. dirhodum* field populations to sulfoxaflor. Specifically, a standard leaf-dipping bioassay was conducted to assess the sulfoxaflor resistance of 24 *S. miscanthi*, 24 *R. padi*, and 10 *M. dirhodum* field populations collected from different regions in China in 2019 and 2021. Additionally, the differences in the toxicity levels of sulfoxaflor between two wheat aphid species in the same area were calculated. The study results provide new insights into the mechanisms mediating the development of insecticide resistance as well as valuable information regarding the rational use of sulfoxaflor.

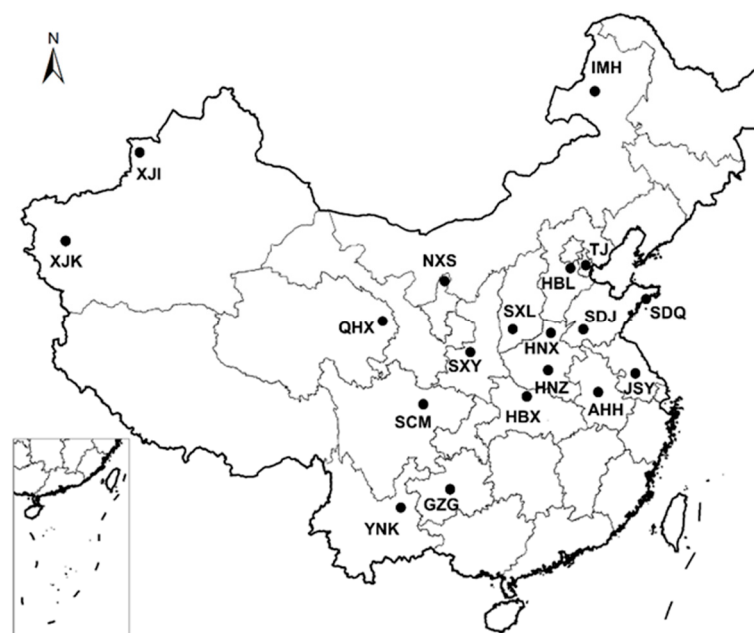
## 2. Materials and Methods

### 2.1. Insects and Insecticides

In 2019 and 2021, *S. miscanthi*, *R. padi*, and *M. dirhodum* field populations were collected from different wheat-producing areas in China (Figure 1, Table S1). The populations in each area were collected from at least three sites, with intervals of more than 10 km between sites. Sulfoxaflor (96%) was supplied by Hubei Kangbaotai Fine Chemicals Co., Ltd. (Wuhan, Hubei Province, China).

### 2.2. Bioassays

The toxicity of sulfoxaflor to aphids was determined using a leaf-based insecticide bioassay method [18]. The insecticide was prepared as a 1% stock solution using acetone. It was then diluted in water (containing 0.1% Tween-80) to produce five working solutions with different concentrations. Water (supplemented with 0.1% Tween-80) was used as the control solution. Wheat leaves containing apterous aphids were dipped in the working (or control) solution for 3–5 s and then placed in a Petri dish with a layer of wet filter paper on the bottom. The Petri dish was placed in an incubator at  $20 \pm 1$  °C with a 16 h light/8 h dark photoperiod and 60–80% relative humidity. At least 30 aphids were treated at each concentration, with three replicates. Mortality was determined using a stereomicroscope after 24 h. Aphids were considered dead if they were unable to move after being touched with an anatomical needle.



**Figure 1.** Wheat aphid sampling regions in China. The sampling regions included Xining, Qinghai (QHX); Shizuishan, Ningxia Hui Autonomous Region (NXS); Hailar, Inner Mongolia Autonomous Region (IMH); Yangling, Shanxi (SXY); Linfen, Shanxi (SXL); Xiangyang, Hubei (HBX); Yangzhou, Jiangsu (JSY); Hefei, Anhui (AHH); Xinxiang, Henan (HNX); Zhumadian, Henan (HNZ); Qingdao, Shandong (SDQ); Jining, Shandong (SDJ); Kashgar Prefecture, Xinjiang Uygur Autonomous Region (XJK); Ili Kazak Autonomous Prefecture, Xinjiang Uygur Autonomous Region (XJI); Langfang, Hebei (HBL); Tianjin (TJ); Mianyang, Sichuan (SCM); Kunming, Yunnan (YNK); and Guiyang, Guizhou (GZG).

### 2.3. Data Analysis

The slopes, 95% confidence limits, and median lethal concentrations ( $LC_{50}$ ) were calculated using PoloPlus 2.00 (LeOra Software Inc., Petaluma, CA, USA). The relative resistance ratio (RLR) was calculated on the basis of the  $LC_{50}$  for the most susceptible field population. Resistance levels were classified as follows:  $5 < RLR \leq 10$  (low resistance);  $10 < RLR \leq 100$  (moderate resistance); and  $RLR > 100$  (high resistance).

The differences in sulfoxaflor toxicity levels between wheat aphid species (i.e., *S. miscanthi* and *R. padi*, *S. miscanthi* and *M. dirhodum*, and *R. padi* and *M. dirhodum*) in the same region were assessed by the 95% confidence intervals of median lethal concentrations ratio [ $LCR_{50}$  (95%CI)] [19] using PoloPlus 2.0. The  $LCR_{50}$  (95%CI)  $> 1$  indicated that the latter species was more susceptible to sulfoxaflor than the former species. The  $LCR_{50}$  (95%CI)  $< 1$  indicated that the latter species was less susceptible to sulfoxaflor than the former species. The inclusion of 1 in the  $LCR_{50}$  (95%CI) indicated that the susceptibility levels of the two species to sulfoxaflor were not significantly different.

## 3. Results

### 3.1. Susceptibility of *S. miscanthi* Field Populations to Sulfoxaflor

An examination of the susceptibility levels of 24 *S. miscanthi* field populations to sulfoxaflor (Table 1) identified HBL-2019 as the most susceptible field population ( $LC_{50} = 2.28$  mg/L; i.e., baseline value). The YNK-2019 and YNK-2021 field populations were highly resistant to sulfoxaflor (RLRs of 194.32 and 110.84, respectively). In total, 13 field populations (SXL-2019, SXY-2019, SXY-2021, HBX-2021, SDJ-2019, AHH-2019, IMH-2019, QHX-2019, HNZ-2019, XJK-2019, GZG-2021, HNX-2019, and NXY-2019) were moderately resistant to sulfoxaflor (RLRs of 10.27–56.18), whereas six field populations (GZG-2019, TJ-2019, SDQ-2019, HNX-2021, HBL-2021, and QHX-2021) exhibited low resistance to sulfoxaflor

(RLRs of 5.52–9.75). Only three field populations (HBX-2019, AHH-2021, and HBL-2019) were not significantly resistant to sulfoxaflor (RLRs < 5).

**Table 1.** Toxicity of sulfoxaflor to *Sitobion miscanthi* field populations.

| No. | Code     | N <sup>a</sup> | Slope ± SE <sup>b</sup> | LC <sub>50</sub> <sup>c</sup> | 95%CL <sup>d</sup> | χ <sup>2</sup> | RLR <sup>e</sup> |
|-----|----------|----------------|-------------------------|-------------------------------|--------------------|----------------|------------------|
| 1   | YNK-2019 | 574            | 0.49 ± 0.09             | 443.05                        | 192.13–1717.55     | 1.26           | 194.32           |
| 2   | YNK-2021 | 664            | 0.88 ± 0.08             | 252.72                        | 179.64–355.16      | 0.48           | 110.84           |
| 3   | SXL-2019 | 451            | 0.64 ± 0.08             | 128.10                        | 76.02–235.77       | 1.52           | 56.18            |
| 4   | SXY-2019 | 582            | 0.64 ± 0.07             | 81.76                         | 25.75–289.65       | 5.82           | 35.86            |
| 5   | SXY-2021 | 866            | 1.39 ± 0.09             | 75.81                         | 38.34–149.07       | 8.91           | 33.25            |
| 6   | HBX-2021 | 831            | 0.96 ± 0.07             | 60.94                         | 46.31–79.29        | 1.61           | 26.73            |
| 7   | SDJ-2019 | 664            | 1.28 ± 0.15             | 58.51                         | 44.71–80.62        | 1.16           | 25.66            |
| 8   | AHH-2019 | 607            | 0.79 ± 0.08             | 57.49                         | 12.71–158.36       | 7.92           | 25.21            |
| 9   | IMH-2019 | 634            | 0.77 ± 0.07             | 52.17                         | 16.18–139.50       | 7.07           | 22.88            |
| 10  | QHX-2019 | 595            | 0.82 ± 0.08             | 44.33                         | 16.97–112.30       | 5.74           | 19.44            |
| 11  | HNZ-2019 | 418            | 0.90 ± 0.10             | 37.34                         | 10.95–140.16       | 7.02           | 16.38            |
| 12  | XJK-2019 | 759            | 0.81 ± 0.08             | 32.27                         | 21.07–46.93        | 1.29           | 14.15            |
| 13  | GZG-2021 | 648            | 0.90 ± 0.07             | 29.18                         | 8.93–66.24         | 7.39           | 12.8             |
| 14  | HNX-2019 | 454            | 0.84 ± 0.09             | 28.83                         | 18.85–43.35        | 2.42           | 12.64            |
| 15  | NXY-2019 | 535            | 0.76 ± 0.08             | 23.42                         | 15.13–34.78        | 2.27           | 10.27            |
| 16  | GZG-2019 | 466            | 0.81 ± 0.08             | 22.24                         | 11.27–42.62        | 3.43           | 9.75             |
| 17  | TJ-2019  | 496            | 0.68 ± 0.09             | 16.40                         | 9.2–26.70          | 1.19           | 7.19             |
| 18  | SDQ-2019 | 552            | 0.69 ± 0.08             | 16.38                         | 9.57–26.64         | 0.87           | 7.18             |
| 19  | HNX-2021 | 805            | 0.66 ± 0.06             | 15.44                         | 8.36–24.86         | 1.07           | 6.77             |
| 20  | HBL-2021 | 555            | 0.84 ± 0.08             | 12.91                         | 8.73–18.67         | 0.94           | 5.66             |
| 21  | QHX-2021 | 700            | 1.11 ± 0.08             | 12.59                         | 7.21–21.72         | 4.62           | 5.52             |
| 22  | HBX-2019 | 575            | 0.56 ± 0.06             | 10.91                         | 4.34–23.28         | 3.04           | 4.79             |
| 23  | AHH-2021 | 797            | 0.62 ± 0.06             | 10.73                         | 3.58–22.28         | 3.10           | 4.71             |
| 24  | HBL-2019 | 332            | 0.53 ± 0.12             | 2.28                          | 0.37–5.65          | 1.54           | 1                |

<sup>a</sup> number of tested aphids. <sup>b</sup> standard error. <sup>c</sup> concentrations (mg/L) resulting in 50% dead or affected after 24 h. <sup>d</sup> 95% confidence limit of median lethal concentrations. <sup>e</sup> relative resistance ratio; 5 < RLR ≤ 10 (low resistance), 10 < RLR ≤ 100 (moderate resistance), and RLR > 100 (high resistance).

### 3.2. Susceptibility of *R. padi* Field Populations to Sulfoxaflor

An analysis of the susceptibility levels of 24 *R. padi* field populations to sulfoxaflor (Table 2) revealed that JSY-2019 was the most susceptible field population (LC<sub>50</sub> = 2.53 mg/L; i.e., baseline value). In contrast, HNX-2021 was highly resistant to sulfoxaflor (RLR of 113.93). Nine field populations (IMH-2019, SDJ-2019, HBX-2021, XJK-2019, SXY-2019, QHX-2021, AHH-2021, AHH-2019, and GZG-2021) were moderately resistant to sulfoxaflor (RLRs of 10.39–40.98), whereas seven field populations (SXL-2019, SCM-2019, SXL-2021, TJ-2019, QHX-2019, SXY-2021, and HNZ-2019) exhibited low resistance to sulfoxaflor (RLRs of 5.02–9.38). Seven field populations (YNK-2019, HBL-2021, NXY-2019, XJI-2021, GZG-2019, HBX-2019, and JSY-2019) were not significantly resistant to sulfoxaflor (RLRs < 5).

### 3.3. Susceptibility of *M. dirhodum* Field Populations to Sulfoxaflor

An evaluation of the susceptibility levels of 10 *M. dirhodum* field populations to sulfoxaflor (Table 3) indicated that XJK-2019 was the most susceptible field population (LC<sub>50</sub> = 1.22 mg/L; i.e., baseline value). The XJI-2021 and SXL-2019 field populations were highly resistant to sulfoxaflor (RLRs of 206.26 and 101.45, respectively). Four field populations (SXY-2021, HBL-2019, SXY-2019, and NXY-2019) were moderately resistant to sulfoxaflor (RLRs of 11.89–68.74), whereas two field populations (GZG-2019 and QHX-2019) exhibited low resistance to sulfoxaflor (RLRs of 7.48 and 6.59, respectively). Two field populations (GZG-2021 and XJK-2019) were not significantly resistant to sulfoxaflor (RLRs < 5).

**Table 2.** Toxicity of sulfoxaflor to *Rhopalosiphum padi* field populations.

| No. | Code     | N <sup>a</sup> | Slope $\pm$ SE <sup>b</sup> | LC <sub>50</sub> <sup>c</sup> | 95%CL <sup>d</sup> | $\chi^2$ | RLR <sup>e</sup> |
|-----|----------|----------------|-----------------------------|-------------------------------|--------------------|----------|------------------|
| 1   | HNX-2021 | 881            | 0.82 $\pm$ 0.06             | 288.24                        | 164.09–506.74      | 4.28     | 113.93           |
| 2   | IMH-2019 | 927            | 0.63 $\pm$ 0.06             | 103.68                        | 44.12–315.22       | 5.88     | 40.98            |
| 3   | SDJ-2019 | 854            | 0.82 $\pm$ 0.10             | 99.45                         | 68.87–155.84       | 1.64     | 39.31            |
| 4   | HBX-2021 | 964            | 0.54 $\pm$ 0.05             | 94.56                         | 61.90–153.76       | 1.58     | 37.38            |
| 5   | XJK-2019 | 662            | 0.86 $\pm$ 0.08             | 93.98                         | 64.19–138.55       | 0.75     | 37.15            |
| 6   | SXY-2019 | 732            | 0.60 $\pm$ 0.07             | 75.04                         | 46.29–121.92       | 1.76     | 29.66            |
| 7   | QHX-2021 | 890            | 1.40 $\pm$ 0.11             | 50.99                         | 21.63–93.13        | 8.64     | 20.15            |
| 8   | AHH-2021 | 670            | 1.28 $\pm$ 0.10             | 34.42                         | 27.64–47.00        | 2.53     | 13.61            |
| 9   | AHH-2019 | 754            | 0.87 $\pm$ 0.07             | 30.31                         | 15.02–54.19        | 4.11     | 11.98            |
| 10  | GZG-2021 | 807            | 1.054 $\pm$ 0.08            | 26.30                         | 18.79–35.17        | 2.66     | 10.39            |
| 11  | SXL-2019 | 666            | 0.65 $\pm$ 0.07             | 23.74                         | 7.35–54.52         | 4.12     | 9.38             |
| 12  | SCM-2019 | 656            | 0.75 $\pm$ 0.07             | 20.34                         | 13.25–30.21        | 2.66     | 8.04             |
| 13  | SXL-2021 | 935            | 1.62 $\pm$ 0.12             | 16.93                         | 13.85–20.32        | 1.20     | 6.69             |
| 14  | TJ-2019  | 629            | 0.74 $\pm$ 0.07             | 15.50                         | 6.45–30.54         | 3.50     | 6.13             |
| 15  | QHX-2019 | 676            | 0.62 $\pm$ 0.07             | 15.13                         | 9.02–23.45         | 2.05     | 5.98             |
| 16  | SXY-2021 | 820            | 1.13 $\pm$ 0.08             | 12.97                         | 6.45–22.90         | 6.14     | 5.13             |
| 17  | HNZ-2019 | 909            | 0.90 $\pm$ 0.06             | 12.69                         | 4.26–30.11         | 10.35    | 5.02             |
| 18  | YNK-2019 | 654            | 0.77 $\pm$ 0.07             | 12.22                         | 5.07–25.24         | 4.99     | 4.83             |
| 19  | HBL-2021 | 727            | 0.95 $\pm$ 0.08             | 9.86                          | 6.51–14.03         | 1.71     | 3.9              |
| 20  | NXY-2019 | 579            | 1.15 $\pm$ 0.11             | 6.74                          | 4.60–9.20          | 1.26     | 2.66             |
| 21  | XJI-2021 | 639            | 1.23 $\pm$ 0.12             | 4.98                          | 3.31–6.91          | 0.36     | 1.97             |
| 22  | GZG-2019 | 587            | 0.76 $\pm$ 0.08             | 4.22                          | 0.57–11.52         | 6.48     | 1.67             |
| 23  | HBX-2019 | 590            | 0.79 $\pm$ 0.09             | 3.75                          | 1.91–6.19          | 1.08     | 1.48             |
| 24  | JSY-2019 | 688            | 0.39 $\pm$ 0.07             | 2.53                          | 0.44–6.83          | 0.07     | 1                |

<sup>a</sup> number of tested aphids. <sup>b</sup> standard error. <sup>c</sup> concentrations (mg/L) resulting in 50% dead or affected after 24 h. <sup>d</sup> 95% confidence limit of median lethal concentrations. <sup>e</sup> relative resistance ratio; 5 < RLR  $\leq$  10 (low resistance), 10 < RLR  $\leq$  100 (moderate resistance), and RLR > 100 (high resistance).

**Table 3.** Toxicity of sulfoxaflor to *Metopolophium dirhodum* field populations.

| No. | Code     | N <sup>a</sup> | Slope $\pm$ SE <sup>b</sup> | LC <sub>50</sub> <sup>c</sup> | 95%CL <sup>d</sup> | $\chi^2$ | RLR <sup>e</sup> |
|-----|----------|----------------|-----------------------------|-------------------------------|--------------------|----------|------------------|
| 1   | XJI-2021 | 652            | 0.60 $\pm$ 0.08             | 251.64                        | 73.00–251.44       | 1.03     | 206.26           |
| 2   | SXL-2019 | 663            | 0.60 $\pm$ 0.08             | 123.77                        | 146.00–502.84      | 1.03     | 101.45           |
| 3   | SXY-2021 | 594            | 0.74 $\pm$ 0.07             | 83.86                         | 38.28–183.91       | 3.90     | 68.74            |
| 4   | HBL-2019 | 864            | 1.36 $\pm$ 0.16             | 25.12                         | 13.39–42.01        | 4.41     | 20.59            |
| 5   | SXY-2019 | 685            | 0.51 $\pm$ 0.06             | 15.72                         | 8.02–27.11         | 2.18     | 12.89            |
| 6   | NXY-2019 | 696            | 0.88 $\pm$ 0.07             | 14.51                         | 10.06–20.33        | 1.60     | 11.89            |
| 7   | GZG-2019 | 586            | 0.78 $\pm$ 0.08             | 9.12                          | 2.69–1.22          | 10.4     | 7.48             |
| 8   | QHX-2019 | 493            | 0.70 $\pm$ 0.10             | 8.04                          | 4.45–12.92         | 1.78     | 6.59             |
| 9   | GZG-2021 | 751            | 1.05 $\pm$ 0.23             | 1.28                          | 0.14–3.18          | 0.09     | 1.05             |
| 10  | XJK-2019 | 579            | 0.44 $\pm$ 0.06             | 1.22                          | 0.08–4.92          | 4.31     | 1                |

<sup>a</sup> number of tested aphids. <sup>b</sup> standard error. <sup>c</sup> concentrations (mg/L) resulting in 50% dead or affected after 24 h. <sup>d</sup> 95% confidence limit of median lethal concentrations. <sup>e</sup> relative resistance ratio; 5 < RLR  $\leq$  10 (low resistance), 10 < RLR  $\leq$  100 (moderate resistance), and RLR > 100 (high resistance).

### 3.4. Differences in the Toxicity of Sulfoxaflor among Various Wheat Aphid Field Populations

*Sitobion miscanthi* and *R. padi*, *S. miscanthi* and *M. dirhodum*, and *R. padi* and *M. dirhodum* field populations simultaneously collected from 20, 9, and 9 regions, respectively, (Table S1) were used to assess the differences in sulfoxaflor toxicity between wheat aphid species. The analyzed differences in the toxicity levels of sulfoxaflor among various wheat aphid field populations, along with LCR<sub>50</sub> (95%CI) values, are shown in Table S2. The susceptibility levels of *S. miscanthi* in 5 of 20 regions, *S. miscanthi* in 3 of 9 regions, and *R. padi* in 3 of 9 regions to sulfoxaflor were not significantly different compared with those of *R. padi*, *M. dirhodum*, and *M. dirhodum* in the same regions, respectively (Table 4). However, *R. padi* in 9 of 20 regions, *M. dirhodum* in 5 of 9 regions, and *M. dirhodum* in 3 of 9 regions were more susceptible to sulfoxaflor than *S. miscanthi*, *S. miscanthi*, and *R. padi* in the same regions, respectively (Table 4). In addition, *R. padi* in 6 of 20 regions, *M. dirhodum* in 1 of 9 regions,

and *M. dirhodum* in 3 of 9 regions were less susceptible to sulfoxaflor than *S. miscanthi*, *S. miscanthi*, and *R. padi* in the same regions, respectively (Table 4). In summary, the *R. padi* and *M. dirhodum* field populations were more susceptible to sulfoxaflor than the *S. miscanthi* field populations.

**Table 4.** The differences in sulfoxaflor toxicity between wheat aphid species.

| Species                                  | N <sup>a</sup> | ns <sup>b</sup> | + <sup>c</sup> | – <sup>d</sup> |
|------------------------------------------|----------------|-----------------|----------------|----------------|
| <i>S. miscanthi</i> / <i>R. padi</i>     | 20             | 5               | 9              | 6              |
| <i>S. miscanthi</i> / <i>M. dirhodum</i> | 9              | 3               | 5              | 1              |
| <i>R. padi</i> / <i>M. dirhodum</i>      | 9              | 3               | 3              | 3              |

<sup>a</sup> number of total field populations. <sup>b</sup> number of field populations showing no differences in sulfoxaflor toxicity between wheat aphid species. <sup>c</sup> number of field populations in which the latter species was more susceptible to sulfoxaflor than the former species. <sup>d</sup> number of field populations in which the latter species was less susceptible to sulfoxaflor than the former species.

#### 4. Discussion

Pest control strategies primarily rely on the application of chemical insecticides. During the past 20 years, pesticides, such as pyrethroids, neonicotinoids, and organophosphates, have been widely used to control wheat aphids in the field [20]. However, wheat aphid field populations have become resistant owing to the extensive use of insecticides [8]. Insecticide resistance is increasingly becoming a problem that affects the sustainable production of important agricultural crops worldwide.

Sulfoxaflor is a new highly effective insecticide that has no known cross-resistance with other insecticides [2]. Accordingly, it can be used as a substitute for other pesticides in insecticide resistance management programs [2]. However, we detected two *S. miscanthi*, one *R. padi*, and two *M. dirhodum* field populations highly resistant to sulfoxaflor. Additionally, 13 *S. miscanthi*, 9 *R. padi*, and 4 *M. dirhodum* field populations were moderately resistant to sulfoxaflor. Another six *S. miscanthi*, seven *R. padi*, and two *M. dirhodum* field populations exhibited low-level resistance to sulfoxaflor. Earlier studies revealed the low-level sulfoxaflor resistance of *N. lugens* and *A. gossypii* field populations [16,17]. These sulfoxaflor-resistance findings present new challenges for the effective use of sulfoxaflor, with implications for the commercial value of this insecticide.

The high sulfoxaflor resistance of wheat aphid field populations may not be the result of long-term sulfoxaflor applications because, to the best of our knowledge, imidacloprid and omethoate were used to control wheat aphids in the YNK, HNX, and SXL regions, from 2013 to 2018. This sulfoxaflor resistance of wheat aphids may be related to unknown mechanisms of cross-resistance to imidacloprid or omethoate. Furthermore, insecticides were not applied on the XJI wheat fields. Thus, there may be other ways in which insects develop insecticide resistance. The ‘pre-adaptation hypothesis’ suggests that generalist herbivores are exposed to a greater variety of chemicals during evolution than specialists and that their ability to transport, isolate, and detoxify these compounds may have pre-adapted them to ‘novel’ xenobiotics (e.g., insecticides) [21]. Multiple studies have shown that endosymbiont bacteria influence host resistance to insecticides [22–24]. Hence, the biological mechanisms underlying the development of insecticide resistance and host plant adaptations during evolution may be the same [25]. Aphids are generalist herbivores and migratory insects. Whether the observed high-level sulfoxaflor resistance of wheat aphid field populations are related to cross-resistance, endosymbiont bacteria, the pre-adaptation hypothesis, or the migration of resistance genes remains to be determined. Nevertheless, the findings of this study provide new insights into how insects develop resistance to insecticides.

Insecticides can alter species interactions and competition. The resulting changes to the community structure may be favorable for potential secondary pest outbreaks [26]. *Sitobion miscanthi*, *R. padi*, and *M. dirhodum* feed on many of the same crops, including wheat, oats, and barley [12]. The diverse susceptibility levels of different wheat aphid species

co-existing on wheat plants to the same insecticide are important factors to consider when developing effective chemical-based methods for controlling aphids. In a recent study on different aphid species collected in the same area, *R. padi* populations were more sensitive to imidacloprid, beta-cypermethrin, thiamethoxam, chlorpyrifos, and omethoate than *Sitobion avenae* populations, and *S. avenae* populations were more sensitive to avermectin and bifenthrin than *R. padi* populations [8]. In our study, *R. padi* and *M. dirhodum* were more susceptible to sulfoxaflor than *S. miscanthi*. These results suggest that the toxicity of the same insecticide to different wheat aphid species in a given region may vary. This difference will affect the efficacy of sulfoxaflor for controlling aphids in wheat fields.

The present study analyzed the field-evolved resistance of three wheat aphid species (*S. miscanthi*, *R. padi*, and *M. dirhodum*) to sulfoxaflor in various regions of China. The data presented may be relevant to the continued monitoring of wheat aphid insecticide resistance, with important implications for wheat production. Insecticide resistance risk assessments are critical for maintaining the efficacy of pest control measures. Therefore, wheat aphid resistance levels to insecticides in some regions must be carefully monitored. To extend the utility of sulfoxaflor, rotating applications of insecticides having different mechanisms on the basis of resistance monitoring results may be an effective strategy for preventing or delaying the development of wheat aphids resistant to sulfoxaflor.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/article/10.3390/agronomy11112325/s1>, Table S1: Wheat aphid sampling sites and collection dates. Table S2: Differences in the toxicity of sulfoxaflor among various wheat aphid field populations.

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