Cotton Aphid (Hemiptera: Aphididae) Resistance to Afidopyropen in Xinjiang Region, China[1]

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Abstract Concentration-mortality response bioassays were conducted in 2021 to define the toxicity of afidopyropen to field populations of cotton aphids, *Aphis gossypii* Glover (Hemiptera: Aphididae), in Xinjiang region, China. Levels of activity of selected enzymes also were measured. Varying levels of resistance to afidopyropen were detected among aphids from nine major cotton-growing areas in the region. Higher resistance levels were detected in aphids collected from Tumshuk, Alar, and Kurle, with resistance ratios of 4.570, 2.058, and 1.565, respectively. Lower resistance ratios of 0.506, 0.632, and 0.775 were detected in aphids collected from Yinli, Wujiaqu, and Hami, respectively. Biochemical assays showed a highly significant positive correlation of the detoxifying enzymes carboxylesterase and multifunctional oxidase with the level of tolerance, with enzyme activity increasing with the level of tolerance. Glutathione S-transferases and acetylcholinesterase activity in field-collected aphids differed significantly from laboratory colony populations, but their activity did not differ among the field populations. We concluded that cotton aphids in major cotton areas of Xinjiang have not yet developed resistance to afidopyropen and remain sensitive to afidopyropen as indicated by resistance ratios of <5; however, resistance of cotton aphids to afidopyropen in southern Xinjiang is higher than that detected in cotton aphid populations in northern Xinjiang.

Key Words *Aphis gossypii,* afidopyropen, detoxifying enzymes, resistance, cotton aphid

The Xinjiang region of China is one of the main areas of cotton, *Gossypium hirsutum* L., production in the country. Production in that region has gradually shifted to mechanized, input-intensive, labor-saving, high-efficiency planting, cultivation, management, and harvest practices (Fan et al. 2009). These practices, likely coupled with increased monoculture farming, have contributed to elevation of the status of the cotton aphid, *Aphis gossypii* Glover, from a minor to a major pest in Xinjiang, with serious effects on cotton production (Jiang et al. 2020, 2021). Over only one growing season, the amount of cotton infested with cotton aphid in Xinjiang increased from 47.89×10^4 ha in 2019 to 56.91×10^4 ha in 2020 (Yu et al. 2020, Zhang et al. 2020a, Guo et al. 2014).

Cotton aphids feed on plant fluids extracted with their piercing-sucking mouthparts (Yan et al. 2020). Feeding may cause curling of leaves, stunted plant

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growth, and reduced numbers of cotton buds and bolls (Feng et al. 2005, Geng and Liu 2009). Honeydew excreted by the feeding aphids serves as substrate for growth of sooty mold that covers the cotton leaves, reducing absorption of sunlight and, thus, negatively affecting leaf photosynthesis. Cotton aphids also may vector plant viruses that could cause cotton yield loss (Begum et al. 2018).

In the Xinjiang region, 20 to 30 overlapping generations of cotton aphids can occur in cotton fields during a growing season (Chao et al. 2019). In seedling cotton, they can reproduce around every 10 d when the air temperature is suitable (Meng and Li 2001). In general, generations may cycle in only 4 to 5 d, contributing to population outbreaks (Yan et al. 2020).

As a major pest of cotton in Xinjiang, chemical insecticides have been employed to control cotton aphids (Zhang and Liu 2016). However, the long-term and frequent application of a single insecticide or several insecticides with a single mode of action has led to the development of resistance to common insecticides, for example, organophosphates, carbamates, pyrethroids, neonicotinoids, in cotton aphids in Xinjiang. Using the early 1990s as a baseline, the resistance of cotton aphids to imidacloprid was 26.3 and 61.1 times in Aksu and Kuytun areas of Xinjiang, respectively (Cui et al. 2016). In 2018, the resistance of cotton aphid populations to acetamiprid was 53.3, 44.1, and 59.2 times in Kuytun, Kurle, and Wujiaqu areas, respectively, all representing high levels of resistance (Zhao et al. 2018). In 2019, resistance to deltamethrin was increased by 142.4 and 1,805.7 times in Shihezi and Kurle areas, respectively, which were extremely high levels of resistance (Pattima et al. 2019). At present, the cotton aphid populations in Bole, Ili, Wujiaqu, Shihezi, Kuytun, and Kurle areas have developed extremely high resistance to pyrethroid and organophosphorus insecticides, and moderate-to-high resistance to neonicotinoid, avermectin, and carbamate insecticides (Zhang 2020).

Monitoring cotton aphid resistance to insecticides is usually based on the changes in the detoxification enzymes activity in cotton aphid (Guo et al. 2007, Koo et al. 2014). Studies have reported that carboxylesterase (CES) and multifunctional oxidase (MFO) play key roles in the resistance of cotton aphids to acetamiprid (Li et al. 2021), and cytochrome P450 is involved in the resistance of cotton aphids to cyantraniliprole (Song 2020). We have determined that cotton aphid populations in Xinjiang region exhibit CES and acetylcholinesterase (AChE) activities that coincide with levels of resistance to imidacloprid, thus, indicating that these two detoxification enzymes are very likely involved in the development of resistance to imidacloprid in the cotton aphid populations (unpubl. data).

Herein, we have focused on afidopyropen, a natural fermentation product of penicillium and a new type of biological insecticide for controlling cotton aphid (Jiang et al. 2020). Afidopyropen was registered and listed in the market in China in 2019, and it has significantly aided in controlling insects with piercing-sucking mouthparts (Chen et al. 2018, 2019; Qu et al. 2020; Zhang et al. 2020b). In addition, afidopyropen significantly reduces egg hatch, emergence adults, and the amount of honeydew secretion in the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Dae and Sang 2019). It is not toxic to non-target organisms such as German scythe, *Aedes aegypti* (L.), potato beetle, *Tribolium castaneum* (Herbst), and *Musca domestica* L. (Leichter et al. 2013).

Afidopyropen has been applied in cotton fields in some areas of Xinjiang region in China. However, it is yet unknown whether there is cross-resistance of cotton

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aphids to afidopyropen and other insecticides used over the long term to control cotton aphids, or if cotton aphid resistance to afidopyropen changes with application rate and interval. In this study, our goal was to determine the concentration-mortality response of cotton aphids to afidopyropen in nine major cotton-growing areas in Xinjiang (i.e., Alar, Kurle, Tumushuk, Yinli, Bole, Wujiaqu, Kuitun, Shihezi and Hami), to determine the activity of detoxification enzymes and nerve-conducting enzymes in cotton aphids, and to determine the relationship of afidopyropen resistance with these four enzymes. Our ultimate objective was to provide foundational information to support the sustainable management of cotton aphids with efficacious afidopyropen application rates and intervals to guide future studies on the resistance management of the cotton aphid.

Materials and Methods

Aphids to be tested were collected from cotton plants at various dates in June to July 2021 (Table 1). The collections were made at each site by removing leaves from randomly selected plants. The leaves with the aphids were placed in plastic bags for transport to the laboratory. A laboratory colony of cotton aphids had been established in 2017 by collecting aphids from cotton grown at Shihezi University Experimental Site in Xinjiang region. These were maintained on cotton foliage maintained in an environmental chamber at 26 ± 1°C, 45–55% relative humidity (RH), and on a 16h:8h (L:D) photo regime at a light intensity of 12,000 Lux. Aphids from this colony were used as a control for comparison of the field-collected aphids with a population of aphids that would be sensitive to insecticides.

Bioassays. The cotton seedling dipping method was used to determine the median lethal concentration (LC_{50}) of afidopyropen for each cotton aphid population. Cotton seedlings were grown in a hydroponic nutrient solution in an indoor area maintained at 26 ± 1°C, 55–70% RH, and a 16h:8h (L:D) photo regime. Those used in the bioassay were in the two-leaf stage of development. Afidopyropen (96.1% technical grade, BASF SE, Ludwigshafen, Germany) was serially diluted in acetone + Tween 80 (0.01%, v/v) to establish concentrations of 0.01, 0.1, 1, 10, and 100 ppm. Acetone + Tween 80 without afidopyropen was used as the control of 0 ppm.

Thirty similar-sized wingless adult aphids were transferred to individual cotton seedlings, and each seedling was then immersed in the appropriate chemical solution for 5 s. Each of the six treatments was replicated three times. All treated cotton seedlings were maintained at 26 ± 1°C, 55–70% RH, and a 16h:8h (L:D) photo regime. Mortality of cotton aphids was assessed 72 h after dipping by gently touching the tarsus and antennae of the cotton aphids with a brush; no movement or response to the touches indicated death.

Enzymatic activity. CES, MFOs, GSTs, and AChE activity was measured in aphids collected from the nine areas previously listed. At each site, 30 live aphids were removed from randomly selected plants using a brush. These were deposited into a centrifuge tube containing saline solution. Once returned to the laboratory, the aphids were crushed using a tissue homogenizer (Shanghai Leigu Instrument Co., Ltd., Shanghai, China), and the mixture was centrifuged at 3,000 rpm for 10 min in a high-speed refrigerated centrifuge (Shanghai Anting Scientific Instrument Factory,

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Table 1. Cotton aphid collection locations and dates.

Region	Collection Date	Longitude and Latitude
Nankou Town, Regiment 12, Alar City	24 June 2021	N40°48′, E81°19′
Boguqi Town, Regiment 28, Kurle City	18 June 2021	N41°48′, E85°57′
Gaimilik Town, Regiment 49, Tumushuk City	13 June 2021	N39°42′, E78°55′
Yushuzhuangzi Town, Regiment 63, Yinli City	21 July 2021	N43°56′, E80°34′
Mushroom Beach Town, Regiment 82, Bole City	24 July 2021	N44°39′, E82°49′
Tieniu Town, Regiment 101, Wujiaqu City	19 July 2021	N44°14′, E87°31′
Wuwu Xin Town, Regiment 129, Kuitun City	15 July 2021	N44°47′, E84°50′
Huayuan Town, Regiment 143, Shihezi City	13 July 2021	N44°28′, E85°53′
Huoshiquan Town, Red Star Second Field, Hami City	17 July 2021	N42°49′, E93°23′

Shanghai, China). The supernatant was transferred to a 1.5-ml centrifuge tube. Those aphids collected from the laboratory colony and serving as a nonresistant control were prepared in the same manner. Activity of each enzyme was determined according to the instructions with the respective enzyme test kits (Shanghai Yuanye Biological Technology Co., Ltd., Shanghai, China). Using optical density (OD) as measured by a microplate reader (Thermo Fisher Scientific Co., Ltd, Shanghai, China) a standard curve for each enzyme was prepared and the obtained OD value was converted to a vitality value. Determinations were replicated three times for each enzyme.

Statistical analyses. LC_{50}s and associated parameters for the concentration-mortality response bioassays were estimated by probit analysis (Finney 1971). Statistical significance between LC_{50}s of afidopyropen in the aphid populations was determined by overlapping of 95% confidence limits (CL). All other data obtained were subjected to analysis of variance, and treatment means were separated using the least significant difference (*T* test) method. Correlations of LC_{50}s with enzymatic activity also were performed. All calculations were conducted using the Statistical Package for the Social Sciences (SPSS Statistics version 20.0; IBM, Armonk, NY).

Results

Resistance of cotton aphid populations to afidopyropen. The resistance ratio (RR), calculated by dividing the LC_{50} of the potentially resistant population by

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Table 2. Concentration-mortality response (ppm) at 72 h postexposure of nine populations of cotton aphid to afidopyropen compared to a susceptible laboratory colony.

Population	*N*	Slope ± SE	LC_{50}	95% Confidence Limit	R^2	RR*
Lab colony	90	0.50 ± 0.049	0.391	0.209–0.697	0.964	1.000
Alar	90	0.39 ± 0.046	0.805	0.384–1.652	0.959	2.058
Kurle	90	0.53 ± 0.050	0.612	0.345–1.065	0.99	1.565
Tumushuk	90	0.41 ± 0.047	1.787	0.899–3.719	0.983	4.570
Yinli	90	0.433 ± 0.048	0.198	0.089–0.390	0.983	0.506
Bole	90	0.32 ± 0.045	0.423	0.160–0.990	0.958	1.082
Wujiaqu	90	0.30 ± 0.044	0.247	0.079–0.620	0.985	0.632
Kuitun	90	0.24 ± 0.044	0.481	0.132–1.473	0.895	1.230
Shihezi	90	0.46 ± 0.048	0.403	0.203–0.755	0.988	1.031
Hami	90	0.394 ± 0.046	0.303	0.132–0.624	0.993	0.775

* RR = Resistance ratio calculated by dividing the median lethal concentration (LC_{50}) of the population by the LC_{50} of the susceptible laboratory colony.

the LC_{50} of the susceptible laboratory colony, was <5 for each of the nine populations tested indicating that cotton aphids from all nine locations were susceptible to afidopyropen (Table 2). The Tumushuk population exhibited the highest LC_{50} of the nine populations at 1.787 ± 0.89 ppm and, based on nonoverlapping 95% CLs, was the only population of the nine tested that had a significantly greater LC_{50} than that of the susceptible laboratory colony (0.391 ± 0.18). The Tumushuk population also had a significantly higher LC_{50} than that of the Yinli (0.198 ± 0.11), Wujiaqu (0.247 ± 0.17), Hami (0.303 ± 0.17), and Shihezi (0.403 ± 0.20) populations. No other significant differences were detected among the LC_{50}s of the populations (Table 2). The RR values reflected these trends with the Yinli (0.506), Wujiaqu (0.632), Hami (0.775), and Shihezi (1.031) populations being the lowest observed while that of the Tumushuk population was 4.570 (Table 2). These results indicate a higher level of resistance to afidopyropen in cotton aphids in Southern Xinjiang than in Northern Xinjiang, which might be attributed to a greater use of afidopyropen against cotton aphids in the southern parts of Xinjiang.

Enzymatic activity. The carboxylesterase (CES) activity detected in the Tumushuk and Kurle populations was significantly higher than that detected in the Yinli and Hami populations and the susceptible laboratory colony. The latter three did not differ significantly, but CES activity in the Tumushuk population was significantly higher than in the Kurle population (Table 3).

Multifunctional oxidase (MFO) activity in cotton aphids from Tumushuk was significantly higher than that measured in any of the other populations or the susceptible laboratory colony. MFO activity in the remaining eight populations of

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Table 3. Carboxylesterase (CSE) activity in nine populations of cotton aphid in Xinjiang region compared to a laboratory colony.

Population	Mean ± SE Activity*	Activity Ratio**
Lab colony	10.481 ± 0.423c	1.000
Alar	10.710 ± 0.325ab	1.022
Kurle	12.120 ± 0.706b	1.156
Tumushuk	13.747 ± 0.494a	1.312
Yinli	10.372 ± 0.793c	0.990
Bole	11.030 ± 0.161ab	1.052
Wujiaqu	11.528 ± 0.0.648ab	1.100
Kuitun	10.763 ± 0.443ab	1.027
Shihezi	10.740 ± 0.107ab	1.025
Hami	10.410 ± 0.281c	0.993

* Means followed by the same lowercase letters are not significantly different (LSD, $P < 0.05$).
** Activity ratio = mean CES activity of population divided by mean CES activity of the laboratory colony.

cotton aphids did not differ significantly from that of the susceptible laboratory colony (Table 4).

Glutathione-S-transferase (GST) activity varied among the populations and the laboratory colony (Table 5) and did not appear related to any observed resistance to afidopyropen. Whether GST plays any role in the cotton aphid metabolism of afidopyropen needs further research.

Acetyl-cholinesterase (AChE) activity was significantly higher in the Alar, Bole, Yinli, and Hami populations than in the susceptible laboratory colony (Table 6). AChE activity in the Kurle, Tumushuk, and Kuitin populations did not differ significantly from that of the susceptible colony, while activity in the Wujiaqu and Shihezi populations was significantly lower than that of the laboratory colony.

Our analysis detected a statistically significant ($P < 0.01$) positive correlation of LC_{50} with CES ($r = 0.844$) and MFO ($r = 0.804$) activity. The correlation analysis showed a negative, but not statistically significant, relationship of LC_{50} with GST ($r = -0.142$) and AChE ($r = -0.134$) activity.

Discussion

The resistance ratio (RR) of each of the nine populations of cotton aphids indicated that the aphids at each of the nine locations were susceptible to afidopyropen (e.g., RR < 5). However, the LC_{50} of afidopyropen against cotton aphids from the Tumushuk location was statistically higher than that of the Yinli, Wujiaqu, Hami, and Shihezi populations, and it was 4.6× greater than that of the susceptible laboratory colony. The LC_{50}s of the Yinli, Wujiaqu, and Hami populations were lower than the LC_{50} of the laboratory colony. We postulate that

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Table 4. Multifunctional oxidase (MFO) activity in nine populations of cotton aphid in Xinjiang region compared to a laboratory colony.

Population	Mean ± SE MFO Activity*	Activity Ratio**
Lab colony	112.359 ± 1.141bcde	1.000
Alar	120.866 ± 3.640bc	1.076
Kurle	125.174 ± 2.601b	1.114
Tumushuk	137.755 ± 3.508a	1.226
Yinli	118.205 ± 1.877bcd	1.052
Bole	120.007 ± 1.956bc	1.068
Wujiaqu	106.015 ± 1.589de	0.944
Kuitun	108.421 ± 0.902cde	0.965
Shihezi	104.693 ± 4.524 e	0.932
Hami	114.044 ± 1.883 bcde	1.015

* Means followed by the same lowercase letters are not significantly different (LSD, $P < 0.05$).
** Activity ratio = mean carboxylesterase (CES) activity of population divided by mean CES activity of the laboratory colony.

Table 5. Glutathione-S-transferase (GST) activity in nine populations of cotton aphid in Xinjiang region compared to a laboratory colony.

Population	Mean ± SE Activity*	Activity Ratio**
Lab colony	70.569 ± 2.926d	1.000
Alar	81.521 ± 1.490a	1.155
Kurle	65.985 ± 0.608e	0.935
Tumushuk	63.355 ± 1.919ef	0.898
Yili	75.327 ± 0.888bc	1.067
Bole	78.866 ± 1.084ab	1.118
Wujiaqu	61.395 ± 0.822fg	0.870
Kuitun	65.810 ± 0.111e	0.933
Shihezi	58.340 ± 0.605g	0.827
Hami	72.630 ± 0.674cd	1.029

* Means followed by the same lowercase letters are not significantly different (LSD, $P < 0.05$).
** Activity ratio = mean carboxylesterase (CES) activity of population divided by mean CES activity of the laboratory colony.

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Table 6. Acetyl-cholinesterase (AChE) activity in nine populations of cotton aphid in Xinjiang region compared to a laboratory colony.

Population	Mean ± SE Activity*	Activity Ratio**
Lab colony	14.077 ± 1.003d	1.000
Alar	18.833 ± 0.316a	1.338
Kurle	13.676 ± 0.658d	0.972
Tumushuk	12.820 ± 0.813de	0.911
Yinli	16.857 ± 0.188bc	1.197
Bole	17.823 ± 0.230ab	1.266
Wujiaqu	12.020 ± 0.174e	0.854
Kuitun	13.739 ± 0.024d	0.976
Shihezi	11.612 ± 0.128e	0.825
Hami	16.050 ± 0.457c	1.140

* Means followed by the same lowercase letters are not significantly different (LSD, $P < 0.05$).
** Activity ratio = mean carboxylesterase (CES) activity of population divided by mean CES activity of the laboratory colony.

this may have been caused the low cotton aphid populations in those production areas during this study as well as the minimal use of afidopyropen in those areas. Furthermore, the observed resistance of cotton aphid populations to afidopyropen was, in general, higher in southern Xinjiang than in northern Xinjiang apparently because of fewer applications and less use of the insecticide in the northern areas of the province.

We also found that CES and MFO activity was higher in those populations of cotton aphids that exhibited higher levels of resistance to afidopyropen. In fact, correlation of LC_{50}s with CES and MFO activity was highly positive ($r = 0.844$ for CES; $r = 0.804$ for MFO). Assays of enzymatic activity are used to detect resistance to insecticides (Zhang and Liu 2020, Zhuo et al. 2008). These are usually CES, MFOs, GSTs, and AChE (Liu et al. 2010, Xu et al. 2020). For example, in cotton bollworm, *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae), CES and MFO activity was prominent in populations displaying low levels of resistance indoxacarb, while CES, MFO, and GST activity was observed in populations with moderate levels of resistance. No discernable differences in AChE activity were found among susceptible, resistant, and moderately resistant bollworms (Wang et al. 2017). Cui et al. (2016) reported that cotton aphids in Beijing Haidian were susceptible to imidacloprid, while cotton aphids in Xinjiang Kuitun and Aksu were moderately resistant, and that CES, MFO, and GST activity was significantly higher in cotton aphids from the Xinjiang Kuitun and Aksu regions than those from Beijing Haidian. We report herein a similar enzymatic response of cotton aphids to afidopyropen with higher levels of CES and MFO activity in those cotton aphid populations showing

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higher levels of resistance to the chemical. GST and AChE activity did not differ among the various cotton aphid populations.

While these results should not cause an immediate alarm of development of resistance by cotton aphids to afidopyropen in the Xinjiang region of China, plans to mitigate and avoid resistance development should be developed and implemented.

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