



Figure S1. Mycelial growth of three pydiflumetofen-resistant mutants of *F. graminearum* and their sensitive parental isolates.

<i>FgSdhA</i>	520	530	540	550	820	830	840	850	860
S27-3.seq	AGCTCTTCCCTACCCGATCTC	C	CACTGTGCGCCGCTC		CAGTCAAAGGAGTAC	GGAAAGGGTGGTCAGGCCTACCGATGCTG			
S2-4-2.seq	AGCTCTTCCCTACCCGATCTC	C	CACTGTGCGCCGCTC		CAGTCAAAGGAGTAC	GGAAAGGGTGGTCAGGCCTACCGATGCTG			
GY-1R.seq	AGCTCTTCCCTACCCGATCTC	G	CACTGTGCGCCGCTC		CAGTCAAAGGAGTTT	GGAAAGGGTGGTCAGGCCTACCGATGCTG			
S27-3R.seq	AGCTCTTCCCTACCCGATCTC	C	CACTGTGCGCCGCTC		CAGTCAAAGGAGTTT	GGAAAGGGTGGTCAGGCCTACCGATGCTG			
S2-4-2R.seq	AGCTCTTCCCTACCCGATCTC	C	CACTGTGCGCCGCTC		CAGTCAAAGGAGTTT	GGAAAGGGTGGTCAGGCCTACCGATGCTG			
GY-1R.seq	AGCTCTTCCCTACCCGATCTC	C	CACTGTGCGCCGCTC		CAGTCAAAGGAGTAC	GGAAAGGGTGGTCAGGCCTACCGATGCTG			
Consensus	agctcttccctacccgatctc	c	cactgtgcgcgcctc		cagtcaaaggagt	ggaaaggggtggtcaggcctaccgatgctg			

<i>FgSdhB</i>	80	90	100	110	120	130	140	150
S27-3.seq	TGTCCTTCACTCGCTCTATGGCCTCGGTCAGCGAACCTGCCAAGG	G	CCCCGAGTCCAAGTTGAAGTCCTTCCAGATCTACCGA					
S2-4-2.seq	TGTCCTTCACTCGCTCTATGGCCTCGGTCAGCGAACCTGCCAAGG	G	CCCCGAGTCCAAGTTGAAGTCCTTCCAGATCTACCGA					
GY-1R.seq	TGTCCTTCACTCGCTCTATGGCCTCGGTCAGCGAACCTGCCAAGG	G	CCCCGAGTCCAAGTTGAAGTCCTTCCAGATCTACCGA					
S27-3R.seq	TGTCCTTCACTCGCTCTATGGCCTCGGTCAGCGAACCTGCCAAGG	G	CCCCGAGTCCAAGTTGAAGTCCTTCCAGATCTACCGA					
S2-4-2R.seq	TGTCCTTCACTCGCTCTATGGCCTCGGTCAGCGAACCTGCCAAGG	G	CCCCGAGTCCAAGTTGAAGTCCTTCCAGATCTACCGA					
GY-1R.seq	TGTCCTTCACTCGCTCTATGGCCTCGGTCAGCGAACCTGCCAAGG	G	CCCCGAGTCCAAGTTGAAGTCCTTCCAGATCTACCGA					
Consensus	tgtcttcaactcgctctatggcctcgggtcagcgaaacctgccaaagg	g	ccccgagtccaagttgaagtccttccagatctaccga					

<i>FgSdhC</i>	270	280	370	380	390	650	660	670
S27-3.seq	GGCCAC	CAGATCCTCGTTA	TATCACC	GGCTGTACACTCTCCG	CACCCCTC	GGCTATTGGTCTGGTTG	CTTTTCCTGTAA	
S2-4-2.seq	GGCCAC	CAGATCCTCGTTA	TATCACC	GGCTGTACACTCTCCG	CACCCCTC	GGCTATTGGTCTGGTTG	CTTTTCCTGTAA	
GY-1R.seq	GGCCAC	CAGATCCTCGTTA	TATCACC	GGCTGTACACTCTCCG	CACCCCTC	GGCTATTGGTCTGGTTG	CTTTTCCTGTAA	
S27-3R.seq	GGCCAC	CAGATCCTCGTTA	TATCACC	GGCTGTACACTCTCCG	CACCCCTC	GGCTATTGGTCTGGTTG	CTTTTCCTGTAA	
S2-4-2R.seq	GGCCAC	CAGATCCTCGTTA	TATCACC	GGCTGTACACTCTCCG	CACCCCTC	GGCTATTGGTCTGGTTG	CTTTTCCTGTAA	
GY-1R.seq	GGCCAC	CAGATCCTCGTTA	TATCACC	GGCTGTACACTCTCCG	CACCCCTC	GGCTATTGGTCTGGTTG	CTTTTCCTGTAA	
Consensus	ggcca	cagatcctcgtta	tatcacccggt	tacactctccg	caccctc	ggctattgggtctgggtt	tttcctgtaa	

Figure S2. Multiple sequence of the *FgSdhA*, *FgSdhB*, and *FgSdhC* genes in pydiflumetofen-resistant mutants of *F. graminearum* and their sensitive parental isolates.

Table S1. Fungicides used in the current study.

Fungicides				Source
Common names	Fungicide groups	Mode of action target	FRAC ^N code	
98.0% Pydiflumetofen	Respiration	SDHI (Succinate dehydrogenase inhibitors)	7	Syngenta Investment Co., Ltd., China
96.2% Tebuconazole	DMI (14 α -demethylase inhibitor)	Sterol biosynthesis in membranes	3	Sheyang Huanghai Pesticide Chemical Co. Ltd.
96.0% Fludioxonil	Phenylpyrrole	MAP/Histidine-Kinase in osmotic signal transduction	12	Hubei Jianyuan Chemical Co. Ltd.
95% Fluazinam	Respiration	Uncouplers of oxidative phosphorylation	29	Zhejiang Heben Pesticide & Chemicals Co. Ltd.
97.5% Pyraclostrobin	Respiration	Complex III: cytochrome bc ₁ (ubiquinol oxidase) at Qo site (cyp b gene)	11	Kangbaotai Fine-Chemical Co. Ltd.
95.0% difenoconazole	Sterol biosynthesis in membranes	DMI-fungicides (Demethylation inhibitions) (SBI: Class I) Azole	3	Hubei Jianyuan Chemical Co. Ltd.
98.1% Carbendazim	Methyl benzimidazole carbamate	β -tubulin assembly in mitochondria	1	Haili Guixi Chemical Co. Ltd.

^NFRAC, Fungicide Resistance Action Committee.**Table S2.** Primers used in the current study.

Primers	Sequences (5' to 3')	Application
FgSdhA F	AACCATATCCAACAAAAGTCCTACTC	Target genes amplification
FgSdhA R	AACTCTTACTACAAAGGAGACGCTG	
FgSdhB F	CTAACGGAGGGGATTGTACGGCATT	
FgSdhB R	TAACCCTTCAGATCGCTTATGCCTCC	
FgSdhC F	TACCAGCCCTCTTTGGAGTAACGACG	
FgSdhC R	CTCGTCCAGACTCCTTTCCCTATATC	
FgSdhD F	AACTCTAACTACTCCAAAAAGCCCT	
FgSdhD R	ATACAGATGCCCCGAGTCATAAACCT	
RT-actin-F	GTCCACCTTCCAGCAAATGT	Target genes expression
RT-actin-R	CCCAAAGCTTAGCGTCTGTC	
RT-FgSdhA-F	AGGCTCCCGCTTCCATTAT	
RT-FgSdhA-R	TTCCGTA CTCTTTGACTGACC	
RT-FgSdhB-F	GCCCATCTTACTGGTGGAACT	
RT-FgSdhB-R	GTGGCAACGGTACAGACTCATT	
RT-FgSdhC-F	GCTCTGGCTGCTGGAATCTC	
RT-FgSdhC-R	GCTACCGAACCATGTCTGCTC	
RT-FgSdhD-F	TTGACCGTCGCTCCCTTTG	
RT-FgSdhD-R	TCTCGAACTCGTACAGTCCCAC	