

Table S3 Evolution of WS types from Independent Fuzzy Spreaders

FS ancestor ^a [<i>fuzY</i> mutation]	WS derivative ^b [phenotype]	SBW25Φ2 sensitivity	Mat ^c strength	<i>fuzY</i> sequence
FS53 [<i>fuzW</i> Δ758-896, Δ <i>fuzX</i> , Δ <i>fuzY</i> , <i>fuzZ</i> Δ1-634]	53.1 [fWS]	resistant	--	
FS8 [Δ4 (-1 Fs)]	8.6b [fWS] 8.7a [WS] 8.8a [WS] 8.8b [WS]	sensitive sensitive sensitive sensitive	- ++ ++ ++	Δ4 (-1 Fs), A5T ^d Δ4 (-1 Fs), A5T ^d 3insA, G112T(Glu>STOP) Δ4 (-1 Fs), A5T ^d
FS73 [Δ28-33]	73.2 [fWS]	resistant	--	
FS116 [G112T]	116.7a [fWS]	resistant	--	
FS162 [C154T]	162.3a [fWS]	resistant	-	
FS105 [Δ193 (-1 Fs)]	105.2a [fWS]	resistant	+	
FS104 [268insT (+1 Fs)]	104.1 [fWS] 104.3a [fWS]	resistant resistant	-- --	
FS133 [C288G]	133.2 [fWS]	resistant	-	
FS156b [G352T]	156b.1 [fWS]	resistant	-	
FS123b [G373T]	123b.2 [fWS]	resistant	--	
FS145b [T376A]	145b.1 [fWS]	resistant	+	
FS115 [Δ383 (-1 Fs)]	115.4 [fWS]	resistant	--	
FS108 [432insCCTGGCGG]	108.1a [fWS] 108.1b [fWS] 108.3a [fWS] 108.3b [SM-like] 108.3d [WS]	resistant sensitive sensitive sensitive sensitive	-- + - ++ ++	Δ433-450 (restores wild-type) Δ433-450 (restores wild-type) Δ433-450 (restores wild-type) Δ433-450 (restores wild-type)
FS [T443G]	FS.2 [fWS]	resistant	--	
FS141 [Δ462-463 (-2 Fs)]	141.9a [fWS]	resistant	--	
FS135 [C519G]	135.1 [fWS]	resistant	--	
FS65 [C565T]	65.3 [fWS]	resistant	--	
FS152 [Δ568-569 (-2 Fs)]	152.3 [fWS]	resistant	-	
FS108 [Δ568-569, 568insA (-1 Fs)]	168.2 [fWS]	resistant	-	
FS146 [Δ592 (-1 Fs)]	146.2 [fWS]	resistant	--	

FS ancestor ^a [<i>fuzY</i> mutation]	WS derivative ^b [phenotype]	SBW25Φ2 sensitivity	Mat ^c strength	<i>fuzY</i> sequence
FS161a [Δ_{618} (-1 Fs)]	161a.1 [fWS] 161a.3 [WS] 161a.4 [fWS]	resistant resistant resistant	-- -- --	
FS143 [G620T]	143.3a [WS]	sensitive	++	T620G (restores wild-type)
FS131 [C662T]	131.2 [fWS]	resistant	-	
FS56 [A638G]	56.1 [fWS]	resistant	--	
FS163 [$\Delta_{638-639}$ (-2 Fs)]	163.2 [fWS]	resistant	-	
FS12 [C662A]	12.1 [fWS]	resistant	--	
FS140 [695insC (+1 Fs)]	140.3b [SM-like]	sensitive	--	Δ_{682} _(-1 Fs) 694insC _(+1 Fs) (corrects frameshift)
FS5 [G712A]	5.2 [fWS]	resistant	-	
FS11/a [<i>fuzY</i> $\Delta_{770-1149}$, <i>fuzZ</i> Δ_{1-220}]	117a.2 [fWS]	resistant	--	
FS68 [$\Delta_{775-896}$]	68.1 [fWS]	resistant	-	
FS92 [Δ_{779} (-1 Fs)]	92.2a [fWS] 92.3 [fWS]	resistant resistant	-- -	
FS145a [809ins126nt]	145a.1 [fWS]	resistant	--	
FS3 [824insTC (+2 Fs)]	3.2 [fWS]	resistant	-	
FS129 [$\Delta_{829-832}$]	129.2 [fWS]	resistant	--	
FS101 [T832A]	101.4a [WS] 101.4b [fWS]	resistant resistant	++ --	
FS44 [G857A]	44.3 [WS]	sensitive	++	A857G (restores wild-type)
FS132 [Δ_{864} (-1 Fs)]	132.3 [fWS]	resistant	--	
FS138 [T872C]	138.3 [WS]	sensitive	++	
FS153 [$\Delta_{882-947}$]	153.2 [fWS]	resistant	-	
FS151b [897insCC (+2 Fs)]	151b.2 [fWS]	resistant	--	
FS87 [945insG (+1 Fs)]	87.1 [fWS]	resistant	--	
FS192b [C947A]	192b.1a [fWS] 192b.2a [WS]	resistant resistant	-- ++	
FS191 [951ins Tn <i>oflu2158</i> , <i>oflu4347</i> , <i>oflu5832</i> , or <i>pflu4873</i>]	191.2 [fWS]	resistant	-	

FS ancestor ^a [<i>fuzY</i> mutation]	WS derivative ^b [phenotype]	SBW25Φ2 sensitivity	Mat ^c strength	<i>fuzY</i> sequence
FS111b _[G953A]	111b.1a [fWS]	resistant	--	
	111b.1b [fWS]	resistant	--	
FS126a _[988insT (+1 Fs)]	126a.2 [fWS]	resistant	-	
FS119 _[Δ1039 (-1 Fs)]	119.2 [fWS]	resistant	--	
FS110 _[<i>fuzY</i>Δ1041-1149, <i>fuzZ</i>Δ1-340]	107.3 [fWS]	resistant	--	
FS157 _[C1048T]	157.3a [fWS]	resistant	--	
	157.3b [fWS]	resistant	--	
FS142 _[1059insT (+1 Fs)]	142.2 [fWS]	resistant	--	
FS156a _[C1096T]	156a.1a [fWS]	resistant	--	T1096C (restores wild-type)
	156a.1b [fWS]	sensitive	--	
FS15 _[no mutation in <i>fuzY</i>]	15.1 [fWS]	resistant	-	
FS43 _[no mutation in <i>fuzY</i>]	43.2a [fWS]	resistant	--	
	43.2b [fWS]	resistant	-	
FS45 _[no mutation in <i>fuzY</i>]	45.1a [fWS]	resistant	--	
	45.1b [fWS]	resistant	--	
	45.1c [fWS]	resistant	--	
FS80 _[no mutation in <i>fuzY</i>]	80.1a [fWS]	resistant	--	
	80.1b [fWS]	resistant	+	
FS179 _[no mutation in <i>fuzY</i>]	179.2b [SM-like]	sensitive	no mat	no mutation in <i>fuzY</i>
	179.2b [WS]	sensitive	++	no mutation in <i>fuzY</i>
	179.2c [WS]	sensitive	++	no mutation in <i>fuzY</i>
FS186 _[no mutation in <i>fuzY</i>]	186.2b [WS]	resistant	+	

Fifty-six independent FS mutants representing the range of *fuzY* mutational types were propagated for 3-day periods by serial dilution in spatially structured microcosms until the emergence of colony morphologies resembling WS types. WS derivatives were scored for SBW25Φ2 sensitivity and mat strength [as an ability to hold 2mm glass beads, as previously described (RAINEY and RAINEY 2003)]. We sequenced *fuzY* from all phage sensitive isolates to identify reverting or compensatory mutations. The majority of the derived types produced feeble mats (fWS) and remained phage resistant, indicative of the fact that the *fuzY* mutation had neither been reversed nor compensated for by a change restoring functionality to the *fuz* locus; conversely, derived WS types producing strong mats were usually phage sensitive. Exceptions to this general trend are indicated in bold.

^a Mutant class is indicated by color [FS (grey); aFS (purple); sFS (green)]. Isolates are ordered by position of mutation (5' to 3').

^b The number of 3-day growth periods prior to detection of each WS isolate is indicated by the number following the decimal point in each isolate name (.1, .2 etc).

^c Mat strength: -- collapsed under the weight of < 5 glass beads; - collapsed under the weight of 5-9 glass beads; + held 10-20 glass beads prior to collapse; ++ held >20 glass beads prior to collapse. Note that typical WS mats uncompromised by *fuzY* mutation are scored as ++.

^d The A5T SNP in isolates 8.6b, 8.7a and 8.8b creates an in-frame 'GTG' start codon, circumventing the -1 frameshift (Fs) caused by Δ4.