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## ▶ To cite this version:

Wesley Mair, Francisco Lopez-Ruiz, Gerd Stammler, William Clark, Fiona Burnett, et al.. Proposal for a unified nomenclature for target-site mutations associated with resistance to fungicides. Pest Management Science, 2016, 72 (8), pp.1449-1459. 10.1002/ps.4301. hal-01588038

## HAL Id: hal-01588038 https://hal.science/hal-01588038v1

Submitted on 26 Sep 2017

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Revised: 18 April 2016

(wileyonlinelibrary.com) DOI 10.1002/ps.4301

# Proposal for a unified nomenclature for target-site mutations associated with resistance to fungicides

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## Abstract

Evolved resistance to fungicides is a major problem limiting our ability to control agricultural, medical and veterinary pathogens and is frequently associated with substitutions in the amino acid sequence of the target protein. The convention for describing amino acid substitutions is to cite the wild-type amino acid, the codon number and the new amino acid, using the one-letter amino acid code. It has frequently been observed that orthologous amino acid mutations have been selected in different species by fungicides from the same mode of action class, but the amino acids have different numbers. These differences in numbering arise from the different lengths of the proteins in each species. The purpose of the present paper is to propose a system for unifying the labelling of amino acids in fungicide target proteins. To do this we have produced alignments between fungicide target proteins of relevant species fitted to a well-studied 'archetype' species. Orthologous amino acids in all species are then assigned numerical 'labels' based on the position of the amino acid in the archetype protein.

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Supporting information may be found in the online version of this article.

Keywords: fungicide; target site; mutation; SDHI; Cyp51

## 1 BACKGROUND

Evolved resistance to fungicides is a major problem limiting our ability to control agricultural, medical and veterinary pathogens.<sup>1,2</sup> Research over the last 30 years has often defined the mechanism conferring reduced sensitivity to the fungicide. Many cases of resistance have been ascribed to the activity of efflux pumps<sup>3</sup> or to overexpression of target genes,<sup>4</sup> but the majority are due, at least partly, to substitutions (or indels) in the amino acid sequence of the target protein.

The convention for describing amino acid substitutions is to cite the wild-type amino acid, the codon number and the new amino acid, using the one-letter amino acid code (see Oliver and Hewitt,<sup>5</sup> box 6.1, p. 138 for a description of the system). A well-known example is the alanine (A) for glycine (G) substitution in the cytochrome *b* gene at position 143 conferring resistance to strobilurin fungicides, referred to as G143A.<sup>6</sup> Further alterations can be amino acid deletions designated with a  $\Delta$  and insertions with an 'ins'.

Target-site amino acid substitutions have been described for seven fungicide groups (named here according to the FRAC convention<sup>7</sup>) and their target proteins. These are C3 and cytochrome *b* (Cytb) (Table 1); G1 and two sterol C14-demethylases (paralogues Cyp51A and Cyp51B) (Tables 2

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 Table 1. CvtB. Position number based on alignment to reference
 sequence from Zymoseptoria tritici (NCBI gene accession number AY247413)

Amino acid substitution(s) in archetype	Homologous position in other species
F129L	F129L in PHAKPA
	F129L in PLASVI
	F129L in PYRIOR
	F129L in PYRNTE
	F129L in PYRNTR
	F129L in RHIZSO
G137	G137R in PYRNTR
G143A	G143A in ALTEAL
	G143A in ALTELY
	G143A in ALTESO
	G143A in ALTETO
	G143A in BOTRCI
	G143A in CERCBE
	G143A in COLLGR
	G143A in ERYSGT
	G143A in LEPTNO
	G143A in MICDMA
	G143A in MONGNI
	G143A in MYCOFI
	G143A in MYCORA
	G143A in PLASVI
	G143A in PLEOAL
	G143A in PODOFU
	G143A in PSPECU
	G143A in PYRIOR
	G143A in PYRNTR
	G143A in RHIZSO
	G143A in VENTIN

 
 Table 2.
 Cvp51A. Position number based on alignment to reference
 sequence from Aspergillus fumigatus (Cyp51A) (NCBI gene accession number AF338659)

Amino acid substitution(s) in archetype	Homologous position in other species
N22D	NA
S52T	NA
G54E/K/R/V/W	G54W in ASPEPA
Y68	Y132N in ASPEFL
Q88H	NA
L98H	NA
V101F	NA
Y121F	Y136F in AJELCP
N125I	NA
K133	K197N in ASPEFL
G138C/R/S	NA
Q141H	NA
H147Y	NA
P216L	NA
F219S	NA
M220K/I/T/V	NA
D280	D282E in ASPEFL
M286	M288L in ASPEFL
T289A	NA
S297T	NA
P394L	NA
Y431C	NA
G432S	NA
G434C	NA
T440A	NA
G448S	NA
T470	T469S in ASPEFL
Y491H	NA
F495I	NA

and 3); B1/B2 and b-tubulin (Table 4); C2 and three of the subunits of the succinate dehydrogenase complex (SdhB, SdhC and SdhD) (Tables 5, 6 and 7); H5 and cellulose synthase A3 (CesA3) (Table 8); E3 and the Os1 family (group III) histidine kinase (Os-1, includes Bos1, BcOS1, Daf1, HK1, HIK1 and NIK1<sup>8</sup>) (Table 9); G3 and the 3-keto reductase (Erg27). Where more than one species has been studied, it has frequently been observed that orthologous amino acid mutations have been associated with resistance to fungicides with the same mode of action.

In cases where the proteins are strongly conserved between species, the mutations have identical numbers. For example, the orthologous Cytb G143A mutation has been found in 22 species (Table 1). However in other cases orthologous mutations have different numbers - e.g. Cyp51B amino acid Y137 in Zymoseptoria tritici is orthologous to amino acids numbered from 131 to 145 in different species (Table 3). Similarly, SdhB amino acid H277 in Pyrenophora teres is orthologous to amino acids numbered from 249 to 278 (Table 5). These differences in numbers create unnecessary confusion and obscure the relationships between mutations in different species.

Resistance caused by insertions in promoters and in efflux pumps have a much lower level of homology and so are not considered here.

#### 2 **THE PROPOSAL**

The differences in numbering arise from the different lengths of the fungicide target protein in each species. The purpose of the present paper is to propose a system for unifying the labelling of mutant amino acids in fungicide target proteins. We propose that orthologous amino acids (i.e. ones presumed to be descended from the same amino acid in the common ancestor of these species) are given the same number in all species regardless of the actual position. The advantages of a unified system is that it would be easier to memorise common changes, to determine whether the changes were novel or were repetitions of what has already been seen in other species and to link changes to particular active ingredients. Orthologous mutations would be assigned the same 'mutation label'.

We distinguish between 'mutation labels', which refer to the orthology between proteins from different species, and 'amino

**Table 4.** b-Tubulin. Position number based on alignment to refer

ence sequence from Aspergillus nidulans (benA) (NCBI gene accession

number M17519)



 
 Table 3.
 Cvp51B. Position number based on alignment to reference
 sequence from Zymoseptoria tritici (NCBI gene accession number AY253234) Homologous position Amino acid substitution(s) in archetype in other species T66 A61V in CANDAL C80 S79T in ERYSGT D107V NA F120L in PHAKPA L126 D134G NA V136A/C/G NA Y137F Y132F/H in CANDAL Y131F/H in PHAKPA Y134F in PUCCRT Y136F in ERYSGH Y136F in ERYSGT Y136E in MONIEC Y136F in MYCOFI Y136F in UNCINE Y140F/H in SACCCE Y145F in FILBNF M145I NA K148 K142R in PHAKPA K143E in CANDAL K147Q in ERYSGH V151 **I145F in PHAKPA** D176 K175N in FRYSGT N178S NA S208T NA N284H NA E300 E297K in CERCBE H303Y NA A313G in MYCOFI A311G G312A NA 1333 1330T in CERCBE A379G A381G in MYCOFI 1381V NA P391 P384S in CERCBE A410T S405F in CANDAL G412A NA H430 H399P in ASPEFL D411N in ASPEEL A453 Y459C/D/N/S/P/Δ Y461D in MYCOFI G460D/A G462A in MYCOFI Y461D/H/S F449S in CANDAL Y463D/H/N in MYCOFI G476 G464S in CANDAL

Amino acid Homologous position substitution(s) in archetype in other species H6L/Y H6Y in LEPTNO H6Y in MONIFC Y50N/S Y50N in GIBBFU  $\beta_1$ -tubulin Y50C in GIBBZE  $\beta_2$ -tubulin Y50C in HYPMOD M73 Q73R in GIBBZE  $\beta_2$ -tubulin 0134K NA A165V NA F167Y in CERCBE F167 F167Y in COCHHE F167Y in GIBBZE  $\beta_2$ -tubulin F167Y in NEUSCR F167Y in PENIEX E198D/K/O E198A/G/K/V in BOTRCI E198A in CERCBE E198V in GIBBFU  $\beta_2$ -tubulin E198K/L/Q in GIBBZE  $\beta_2$ -tubulin E198A/O in HELMSO E198A/K in MONIFC E198G in NEUSCR E198A/K in PENIAU E198A/K/V in PENIEX E198K in PENIIT E198A/G in PYRPBR E198G/K in RHYNSE E198A/K in SCLEHO E198A in SCLESC E198A/K in VENTIN F200Y F200Y in BOTRCI F200Y in GIBBFU  $\beta_2$ -tubulin F200Y in GIBBZE  $\beta_2$ -tubulin F200Y in PENIAU **F200Y in PFNIIT** F200Y in RHYNSE F200Y in VENTIN L240 L240F in MONILA L240F in PYRPBR L240F in VENTIN M257L NA

the mutation underlying the amino acid substitution is a random event and of no obvious relevance. Definitively linking a mutation to a sensitivity change can be technically very demanding. If resistance to the same class of fungicide is linked to mutations affecting orthologous codons in different species, this is strong, if still circumstantial, evidence of the importance of the mutation. Unifying the mutant labelling system will make it much easier to identify important codon changes. This would assist the prioritisation of research aiming functionally to characterise mutations.

## **3 OPTIONS FOR PRODUCING THE ALIGNMENTS**

We have produced a set of draft alignments of each target protein for which resistance to multiple species has been reported (Figs 1 to 9) and tables of putatively orthologous amino acids in other species where fungicide resistance has been reported (Tables 1

acid numbering', which remains the order of the amino acids in each protein in each species. To avoid confusion, we propose that mutation labels should be italicised and mutation numbers should use regular lettering.

R479

1483

V490L

T496

G510C

S524T

In several cases, amino acid substitutions have been found in the target protein but have not been definitively associated with any change in sensitivity either *in vitro* or in the field. It may be that

G484S in FII BNF

R467K in CANDAL

I471T in CANDAL

1475T in PHAKPA

NA

T454P in ASPEFL

NA

S508T in PYRPBR

S509T in ERYSGH

Table 5. SdhB. Position number based on alignment to referen	ice
sequence from Pyrenophora teres f. sp. teres (NCBI gene accessi	on
number XM_003302513)	

Amino acid substitution(s) in archetype	Homologous position in other species
P230 N235	P225F/L/T in BOTRCI N225I/T in SEPTTR
	N230I in BOTRCI
H277Y	H249L/N/Y in EUROOR
	H257L in USTIMA
	H267L/R/Y in SEPTTR
	H273Y in SCLESC
	H272L/R/V/Y in BOTRCI
	H277R/Y in ALTEAL
	H277R/Y in ALTESO
	H277R/Y in DIDYBR
	H278R/Y in CORYCA
	$H \rightarrow Y$ in PODOXA <sup>a</sup>
1279	I269V in SEPTTR

<sup>a</sup> The amino acid position number for this substitution is unknown as only a 176 bp fragment of SdhB gene has been sequenced in both sensitive and resistant isolates.

 Table 6.
 SdhC. Position number based on alignment to reference sequence from *Pyrenophora teres* f. sp. *teres* (NCBI gene accession number XM\_003302752)

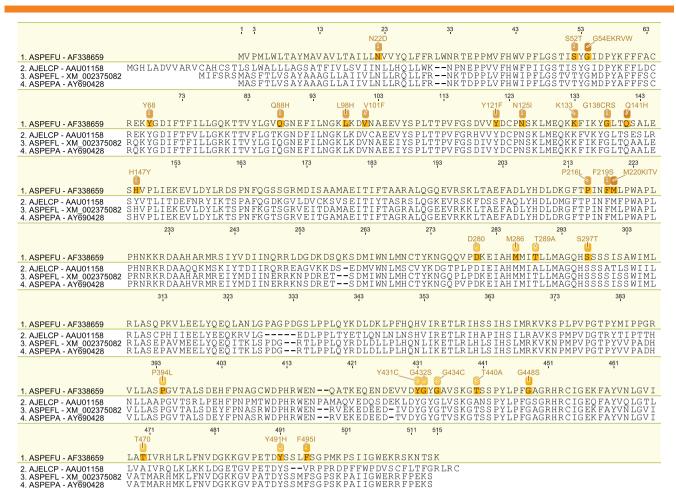
Amino acid substitution(s) in archetype	Homologous position in other species
T68	T79I/N in SEPTTR
W69	W80S in SEPTTR
S73	S73P in CORYCA
	A84V in SEPTTR
	A85V in BOTRCI
N75S	N86K/S in SEPTTR
T78	T90I in EUROOR
G79R	G90R in SEPTTR
H134R	H134R in ALTEAL
	H146R in SCLESC
S135R	NA
H141	H152R in SEPTTR

Table 7. SdhD. Position number based on alignment to reference
sequence from Pyrenophora teres f. sp. teres (NCBI gene accession
number XM_003297196)

Amino acid substitution(s) in archetype	Homologous position in other species
S118	S89P in CORYCA
D124E/N	NA
H134R	H132R in BOTRCI
	H132R in SCLESC
	H133R in ALTEAL
	H133R in ALTESO
G138	G109V in CORYCA
D145G	D124E in EUROOR
	D129E in SEPTTR

1. SEPTTR - AY247413	1         リ         キ         キ         キ         キ         キ         キ         キ         キ         キ         キ         キ         キ         エ         キ         1 <th1< th=""> <th1< th=""> <th1< th=""> <th1< th=""></th1<></th1<></th1<></th1<>
2. ALTEAL - DQ209283 3. ALTELY - AY263413 4. ALTESO - DQ209285 5. ALTETO - AY263411 6. BOTRCI - FJ217744	MRIFKSHPLLKLVNSYMIDSPOPSNLSYLWNFGSLLAVCLVIOIITGVTLA
7. CERCBE - JO619932 8. COLLOR - AY285743 9. ERYSGT - AF343441 10. LEPTNO - ABU49434	SLLAVSLUIQIITGVTLA MRLLKSHPILRLANSYLVDSPQPLNLSYMWNFGSLLGVCLIIQITGVTLA
11. MICDMA - FJ560376 12. MONGNI - FJ560375 13. MYCOFI - AF343070	IIDXPQPSNISYLWNFGSLLGFCLVIQIVTGVTLA
14. MYCORA - JQ859020 15. PLASVI - ABF25501 16. PLEOAL - KJ934233 17. PODOFU - EF058828 18. PSPECU - KF679519	MIRWNKKSLFTVINNHLIDYPTPINLNYFYGFGSLAGIMLVVQILTGIFLA MRIFKSHPFLKLVNAVVIDHSOPTNISYMWNFGSLLGVCLVVOIITGVTLA
18. PSPECU - KF678519 19. PYRIOR - AY245425 20. PYRNTE - DO919007 21. PYRNTR - DO919058 22. RHZSD - AGK45434 23. VENTIN - AF047029	MRIFKSHPFLKLVNAVVIDHSOFTNISYMMNFGSLLGVCLVVOIITGVTLA MRILKSHSLLKLVNSVLIDASOFSNISYLMNFGSLLAVCLVVOIITGTLA MRLLKSHFILGLLNSVMVDSPOPANISYMNFGSLLGMCLVIOIITGAFLA MRILKSHFLLKLNNSVIIDSPÖFSNISYLMNFGSLLAFCUJOIITGAFLA
	NEILKSREDDKDARSTITUSFQFSRISTURREGSLDAFCDFQTTF0/TDA           P         P         P         P           MHYNPSVSEAFNSVEHINR DVNNGWLIR YLHSNTASAFFFLVYLHVGRGLY
2. ALTEAL - DQ209283 3. ALTELY - AY263413 4. ALTESO - DQ209285 5. ALTESO - DQ209285 5. ALTETO - AY263411	IMR DVNNGWLIR YLHSNTASAFFFIV YLHIGRGMY GLY IMB DVNNGWLIR YLHSNTASAFFFIV YLHIGRGMY
6. BOTRCI - FJ217744 7. CERCBE - JQ619932 8. COLLGR - AY285743 9. ERYSGT - AF343441	MHYNPSVLEAFNSVEHÎMÊDVÎNGWLÎRYLHSMYASAPPFIYYLHÎCKGLY BARFFLYVLHYCRGLY DYNGWLIRYLHSMYASAPFFLYVLHYCRGHY HHYNPSVLEAFNSVEHIMEDVÎNGWLIRYLHSMYASAFFFLYVLHICGGLY
1. SEPTIR - AV24743 2. ALTEAL - O209983 3. ALTEL - AV283413 5. ALTEG - AV283413 6. BOTRIC - F287764 6. BOTRIC - F287764 7. CEROSE - JOS19032 5. ERYSGT - AV28543 5. ERYSGT - AV28543 5. ERYSGT - AV28543 5. ERYSGT - AV28543 11. MICOFIA - AV28543 13. MICOFIA - AV28543 13. MICOFIA - AV28543 15. PL/SOL - AU285433 16. PL/SOL - AU285433	INF DVINISKLIR YLHSINTASAFFFI VYLHI GRÖY INF DVINISKLIR YLHSINTASAFFFI VYLHI GRÖY MIYNFSVLEAFNSVEN INF DVINISKLIL SINTASAFFFI VYLHI GGÖY DVINISKLIR UNISKLIL SINTASAFFI VYLHI GGÖY MIYNFSVLEAFNSVEN INF DVINISKLIR INF SINTASAFFI VYLHI MIYNFSVLEAFNSVEN INF DVINISKLIR INF SINTASAFFI VYLHI GGÖY MIYNFSVLEAFNSVEN INF DVINISKLIR INF SINTASAFFI VYLHI GGÖY MIYNFSVLEAFNSVEN INF DVINISKLIR INF SINTASAFFI VYLHI GGÖY MIYNFSVLEAFNSVEN INF DVINISKLIR INF SINTASAFFI VYLHI GGÖY
16. MTCORK - 3026000 15. PLASVI - ABF29001 18. PLEOAL - K.834233 17. PODOFU - EF08828 18. PSPECIL, KF829519	MHYTPHIDLAFNSVERING DVNNGRUIR VLHANGASEFFIVYLHIFRGLY DVNNGRUIR VLHANGASEFFIVYLHIFRGLY MHYNPSVAFAFNSTRHIM DVNNGRUUR VLHANTASAFFFIMVLHIGRGLY
18. PLEOAL - KU934233 17. PODOFU - EF988028 18. PSPECU - KF676519 19. PYROR - A7245425 20. PYRNTE - DQ919067 21. PYRNTR - DQ919060 22. RH200 - A6K45434 23. VENTN - AF647029	DVINCHUY QLIANTASAFFINYLIIGGUY MHYDRSVAEAPNSIEHINGUNGUY QLIANTASAFFIYLYLIIGGUY MHYSRSVMAANNSIEHINGUNGUY QLIANTASAFFIYLYLIIGGUY MHYSRSVMAANNSIEHINGUNGUL AVLIANTASAFFIYLIIGGUY MHYCRSVDATANSEHINGUNYCHANTASAFFIYLIIGGGU
	110 120 130 540 150
1. SEPTER. AV22413 2. Al. TEA. CO20161 3. Al. TEA. CO20161 4. AL TESO. CO20163 5. AL TECO. CO20163 5. AL TECO. CO20163 5. AL TECO. AV23611 6. CO20162 - AV23614 10. LETNO. AV23614 10. LETNO. AV23614 10. LETNO. AV23614 10. LETNO. AV23614 10. LETNO. AV23614 10. LETNO. AV23614 11. MVCORA CO2016 12. MVCORA CO2016 13. MVCORA CO2016 14. PACIDA - CO2016 14. PACID	
1. SEPTTR - AY247413 2. ALTEAL - DQ209283 3. ALTEAL - AY262411	IPWVGQDIVEFVWG-GFSVNNATLNRFFALHFVLPFVLAALVL IPWVGDIVEFIWG-GFSVNNATLNRFFSLHFVLPFVLAALAL
1. SEPTIR - AV26/413 2. AV. TIM-L. OSS9931 3. AV. TELY - AV293413 3. AV TELY - AV293413 5. AV. TESO - D0295025 5. AV17ESO - D0295025 5. AV17ETO - AV293411 7. CERCOBE - J021704 7. CERCOBE - J021704 10. LEPTNO - ABU49431 10. LEPTNO - ABU49431 11. MICDAN - J026370 12. MICORA - AV36370 13. MICORA - AV36370 14. MICORA - AV36370 15. MICORA - AV36370 16. PLEOAL - KR77619 10. PH90R- AV265423 10. PUPDLR - AV265423 10. PUPDLR - D0211007 10. PUPDLR - D021107 10.	
10. LEPTNO - ABU49434 11. MICDMA - FJ580375 12. MONGNI - FJ580375 13. MYCOFI - AF343070 14. MYCOFIA - K04543070	IEWYGODIVEFIWGYCFKWSYQHKCSVNNATLNRFFSLHFVLPFVLAALVL IEWIGODIVE IEWIGODIVE IEWYGODIVESTWG-GLSVNNATLNRFFALHFVFPFVLAALAL IEWYGODIVESTWG-GLSVNNATLNRFFALHFVFPFVLAALAL
15. PLASVI - ABF25501 16. PLEOAL - KJ834233 17. PODOFU - EF088828 18. PSPECU - KF675519	IELIGKEVVDMUNG-GPVDDPTLNPFSLHFTPPVTVGAVL IPWYGODIVEFING-GPSVNNATLNPFSLHFVLPVLAALLU IPWIGDIVEFING-GPSVNNATLNPFPLHFULPVLAALVV VPWIGDVVEFING-GPSVNNATLNPFPLHFULPVLAALVV
19. PYRIOR - AY245425 20. PYRNTE - D0919057 21. PYRNTR - D0919068 22. RHZ50 - AGK45434 23. VENTIN - AF047029	IPWIGQDIVEFIWG-GFSVNNATLNRFFALHFVLPFVLAALVL IPWI IPWIGODFVOLVWG-GFSVNNATLNRFFSLHYLLPFVLAALAL IPWIGQDIVEELWG-GFSVNNATLNRFFALHFVLPFVLAALAL IPIIGQDIVEELWG-GFSVNNATLNRFFALHFVLPFVLAALAL
1. SEPTTR - AY247413 2. ALTEAL - DQ209283	Ref         Rf         Rf           MHLIALHDTAGSGNPLGVSGNYDRLPFAPYFIFKDLITIFLFIIVLSLFFF           MHLIVLHDTAGSGNPLGVSGNYERISFAPYFIFKDLITIFAFIFVLSLFVF
<ol> <li>ALTELY - AY263413</li> <li>ALTESO - DO209285</li> </ol>	MHLIVLHDTAGSGNPLGVSGNYERISFAPYFIFKDLITIFAFIFVLSLFVF
5. ALTETO - AY283411 6. BOTRCI - F1217744 7. CERCBE - J0019932 8. COLLGR - AY285743 9. ERY8GT - AF343441 10. LEPTNO - ABL49434 11. MICDMA - F1960376 12. MIOLAG, EPHY075	NHL LALHDSAGSONDLGISONDDLAPAYYLEKOLTTILETIILSIVF HHL IALHDSGSONDLGYSONDLEPAPYTIKDLTTILITIVSYVP HHL IALHDTGSSNELGYSGNYDR IPPAPYTIKDLTTILTIVSVPGISLEVF HHL IALHDSGSGNPLGSGNYDR IPPAPYTIKDLTTITITITIILISNVP HHL IALHDSGSGNPLGISGNYERIPFAPYFIKDLITIFAFIFVLSLEVF
11. MICDIA - FJ86375 12. MONGNI - FJ86375 13. MYCOFI - A7843070 14. MYCOFI - A7843070 15. PLASVI - A8F28001 16. PLE0AL - K034233 17. PODOFU - EF081028 18. PSPECU - KF876519 19. PYRIOR - AY245425 20. PYRIOR - AY245425	MHL IAL HIGASSIMPL (VSON VDELE PAYFI FINLITTELLIVULSI VP MEL IVLIDVASSIMPL (VSON VDELE PAYFI FINLITVASI VP THE LIVLIDVASSIMPL (VSON VDELE FARAFICE) (VLIPTI FILITVA MEL IVLIDVASSIMPL (VSON VDELE FARAFICE) (VLIPTI FILITVASI MEL IVLIDVASSIMPL (VSON VDELE FARAFICE) (VLIPTI FILITVASI MEL IVLIDVASSIMPL (VSON VDELE FARAFICE) MEL IV
20. PYRNTE - D0919067 21. PYRNTE - D0919067 22. RHZSO - AGK45434 23. VENTIN - AF047029	MHITALHEH-GSSNELGISGNVDRLSFHPFPTEKDLVTVELFLLTLSIFVF MHITALHEB-GSSNELGISGNVDRLSFHPFPTEKDLVTVELFLLTLSIFVF MHITALHESAGSNELGVSGHFORLFFAPTFIKDLITTELFILGISFFVF av av a
1. SEPTTR - AY247413 2. ALTEAL - DQ209283 3. ALTELY - AY203413	FMPNVLGDSENYVMANPMQTPPAIVPEWYLLPFYAILRSIPNKLLGVIAMF FMPNVLGDSENYVVANPMQTPAAIVPEWYLLPFYAILRSIP
	FMPNVLGDSENYVVANPMOTPAAIVPEWYLLPFYAILRSIPNKLLGVIA
<ol> <li>ALTEDO - OLZOBOSO S. ALTETO - AY263411</li> <li>BOTRCI - FJ217744</li> <li>CERCBE - JO619932</li> <li>COLLGR - AY285743</li> <li>ERYSGT - AF343441</li> <li>LEPTNO - ABU49434</li> <li>MCDMA - FJ50338</li> </ol>	FMPNVLGDSDNYIMANPMOTPPAIVPEWYLLPFYAILRSIPNKLLGVIAML FMPNVLGDSDNYVAANPMOTPPAIVEWYLLPFYAILRSIPNKLLGVIAMF FMENVLGDSDNYVAANPMOTPAIVEWYLLPFYAI FMENVLGDSDNYVAANPMOTPAAIVEWYLLPFYAILRSIPNKLLGVIAMF FMENVLGDSDNYVAANPMOTPAAIVEWYLLPFYAILRSIPNKLLGVIAMF
12. MONGNI - F.295075 13. MYCOFI - AF343070 14. MYCOFA - JQ65020 15. PLASVI - AB22501 16. PLEOL - KR34233 17. PODOFU - EF038828 18. PSPECJ - KR767619 19. PYRNTE - DC215087 21. PYRNTE - DC215087 21. PYRNTE - DC215087	IN FUL GOSENIVANIPATION PAIVENUL PAILS EINEL GY AMF FUNNL GOSENIVANIPATION PAIVENUL PAILS EINEL GY AMF FUNNL GOSENIVANIPATION PAIVENUL PAILS EINEL GY FUNNL GOSENIVANIPATION PAIVENUL PAILS EINEL GY MUNIC GOSENIVANIPATION PAIVENUL PAILS EINEL GY MUNIC GOSENIVANIPATION PAIVENUL PAILS EINEL GY AMP
19. PYRIOR - AY245425 20. PYRNTE - DC910057 21. PYRNTR - DC919058 22. RHI2SO - AGK45434 23. VENTIN - AP947029	FNDNULGDSDNYINANPMQTPAATUPEWYLLDFYATIRSIPNKLLGVIANF YMPNALGHSDNYIPANPMSTPASIVPEWDLLPYYAILRSIPNKLUGVIAMF FARNILGDSENYYVANPMQTPPAIVEEWYLLDFYAILRSIPNKLLGVIAMF
1. SEPTTR - AY247413 2. ALTEAL - DQ203283	SAILIIMIMPITDLGRSRGLQFRPLSKITFYIFVANFLVLMQLGANHVESP
2. ALTEAL - DQ209283 3. ALTELY - AY263413 4. ALTESO - DQ209285 5. ALTETO - AY263411 6. BOTRCI - FJ217744	SAILILLAMPFTDLSRSRGIQFRPLSKIAFYIFIANFLILMVLGAKHVESP
5. ALTETO - AY283411 6. BOTRCI - F1217744 7. GERCBE - J0619932 8. COLLER - AY285743 9. ERYSGT - AF343441 10. LEPTNO - ABU9434 11. MICTINO - ABU9434	SA SAILALLAMPFTDLSRSRGLQFKPLNKAAFYIFIGNFVILMVLGAKHVESP SAILILLLPITDVSRSRGMQFRPLSKWAFFVFVANFLILMQLGAKHVESP
11. MICOMA - FUSIOSTIS 12. MONGNI - FUSIOSTIS 13. MYCOFI - AFSH3070 14. MYCORA - JQ859020 15. PLASVI - ABF29501	SAILIIMIMPFTDLGRSRGLQFRPLSKIAFFVFVANFLVLMQXGAKHVESP GSLILLTIPFTNSSEIRSTTFRPIFKVCYMLLVVAFILLGWVGQCPVEYP
16. PLEOAL - KJ834233 17. PODOFU - EF088828 18. PSPECU - KF679519 19. PYRIOR - AY245425	AAILIILALPIVDLGRTQGLQFRPLSKVVFWIFVVNFLILMQLGAKHVESP SAILAIMLLPVTDLGRSRGLQFRPFSKIAFWVFVANFLVLMQLGAKHVEDP
20. PYRNTE - DC919057 21. PYRNTR - DC919068 22. RHZSO - AGK45434 23. VENTIN - AF047029	ASLLILLIMPIVDTSRIRGNOFRPLMKFFFWVFVANFFILMWIGSOHPNSP NAIVILLVMPFTDLGRSGTOFRPLSKIAYYFFIANFLILMKLGARHVESP
1. SEPTTR - AY247413 2. ALTEAL - DO209283	an an ri an FIEFGQISTVLYFSHFLIIVPLVSLIENTLVDMHLNNTIT
2. ALTEAL - DQ209283 3. ALTELY - AY283413 4. ALTESO - DQ209285 5. ALTETO - AY283411 6. BOTRCI - FJ217744	YIEFGQISTVIYFAHFLIIVPFISLLENSLVELAVLTKEKPSR
7. CERCBE - JO619932 8. COLLGR - AY285743 9. ERYSGT - AF343441 10. LEPTNO - ABL49434	YIELGOFSTLLYFSHFLLIVPLVNFLENTLIVLSCRSS FIEFGQISTVLYFLYFTVVMYGYTFIENTFVDLNFYTNYKHSSRFNFVTKK
11. MICDMA - FJ500376 12. MONONI - FJ560375 13. MYCOFI - AF343070 14. MYCORA - JQ856020 15. PLASVI - ABF29501	- YIEFGQIRTVLYFSHFLIIVPLLSILENTLIDLHLNNPK YTEIGIISMIYYFSFFLIIIPFLGKIETYLIRYKCNK
15. PLASVI - ABE25001 16. PLEOAL - KJ034233 17. PODOFU - EF08N828 18. PSPECU - KF678619 19. PVINOR - AY245425 20. PVINOR - AY245425	FIEFGOISTVLYFSHFLIIIPFLGKIETTLIKIKCNK FIEFGOISTVLYFSHFLIIIPITSLIENVLISVNARFOOAQ FILLGQLSTVLYFSYFVAILPLASYLDNSLTDLSNKSELFLNKIN
20. PYRNTE - DQ919067 21. PYRNTR - DQ919088 22. RHIZSO - AGK45434 23. VENTIN - AF647629	- FVEVGQVATALYFAWFVIIVPLVGIVENTLMDLALTSHDS FIEFGQISTVLYFSHFVIIVPLVSLIENTLVDLHLHNTLSLKNVF

**Figure 1.** Amino acid sequence alignment of the Cytb family. Sequences are named by species EPPO code and NCBI gene accession number. Residues highlighted in yellow in the archetype sequence from *Zymoseptoria tritici* denote amino acid substitutions associated with fungicide resistance at an orthologous position in any of the sequences. Numerical mutation labels shown above the alignment are based on the position number of the amino acid in the archetype protein.



**Figure 2.** Amino acid sequence alignment of the Cyp51A family. Sequences are named by species EPPO code and NCBI gene accession number. Residues highlighted in yellow in the archetype sequence from *Aspergillus fumigatus* (Cyp51A) denote amino acid substitutions associated with fungicide resistance at an orthologous position in any of the sequences. Numerical mutation labels shown above the alignment are based on the position number of the amino acid in the archetype protein.

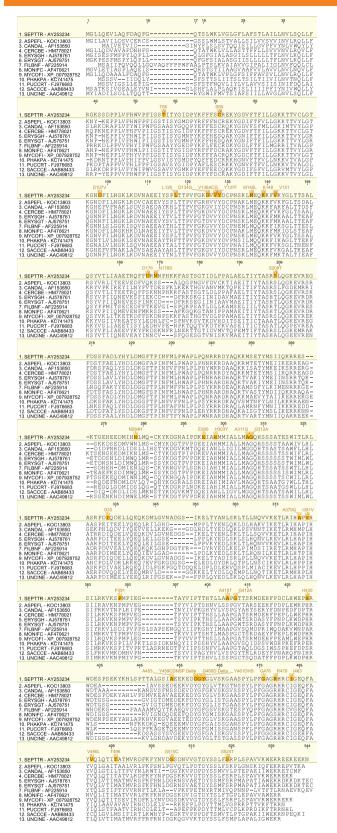
Table 8.         CesA3. Position number based on alignment to reference sequence from <i>Phytophthora infestans</i> (NCBI gene accession number EF563995)				
Amino acid	Homologous position			
substitution(s) in archetype	in other species			
Q1077	Q1077K in PHYTCP			
G1105A/V G1105S/V in PLAS				
	G1105V/W in PSPECU			
V1109L	V1109L/M in PHYTCP			
	V1109L in PHYTDR			

to 9). The species included in these tables and alignments have been referred to by their European and Mediterranean Plant Protection Organisation (EPPO) codes<sup>9</sup> as listed in Table 10.

The alignments for *b*-tubulin and Cytb are essentially colinear in fungi studied to date, and hence there are no changes to be made to the current nomenclature. For the other genes, we have considered four possible methods to generate the alignment. The alignment could be: (1) fitted to the longest gene in the gene set; (2) fitted to a strict consensus alignment; (3) fitted to the gene from the species that is currently the most researched species for 
 Table 9.
 OS-1. Position number based on alignment to reference sequence from *Botrytis cinerea* (Bos1) (NCBI gene accession number AF435964)

Amino acid substitution(s) in archetype	Homologous position in other species
F250	F267L in PLEOAL
1273	L290S in PLEOAL
I365N/R/S	NA
V368F	NA
Q369H/P	NA
N373S	NA
G403	G420D in ALTELO
T447S	NA
E738	E753K in ALTEBI
T750	T765R in PLEOAL
Q762	Q777R in PLEOAL

the fungicide resistance concerned; (4) fitted to the gene from the species that was the first species for the fungicide resistance concerned.



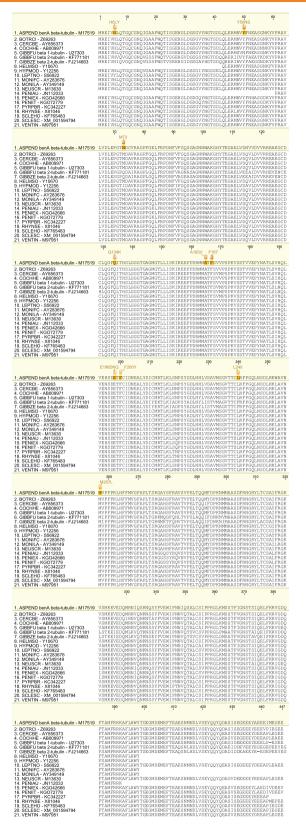
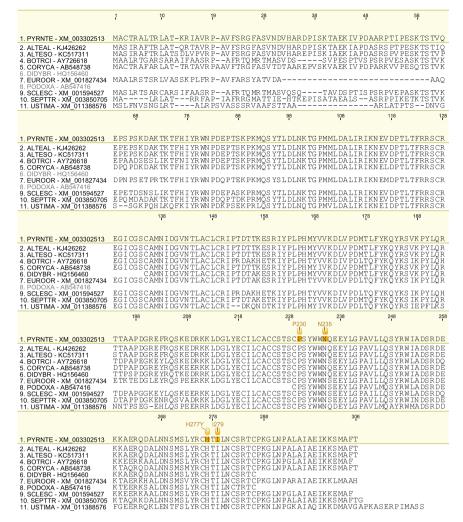


Figure 3. Amino acid sequence alignment of the Cyp51B family. Sequences are named by species EPPO code and NCBI gene accession number. Residues highlighted in yellow in the archetype sequence from Zymoseptoria tritici denote amino acid substitutions associated with fungicide resistance at an orthologous position in any of the sequences. Numerical mutation labels shown above the alignment are based on the position number of the amino acid in the archetype protein.

Figure 4. Amino acid sequence alignment of the *b*-tubulin family. Sequences are named by species EPPO code and NCBI gene accession number. Residues highlighted in yellow in the archetype sequence from Aspergillus nidulans (benA) denote amino acid substitutions associated with fungicide resistance at an orthologous position in any of the sequences. Numerical mutation labels shown above the alignment are based on the position number of the amino acid in the archetype protein.



**Figure 5.** Amino acid sequence alignment of the SdhB family. Sequences are named by species EPPO code and NCBI gene accession number. Residues highlighted in yellow in the archetype sequence from *Pyrenophora teres* f. sp. *teres* denote amino acid substitutions associated with fungicide resistance at an orthologous position in any of the sequences. Numerical mutation labels shown above the alignment are based on the position number of the amino acid in the archetype protein.

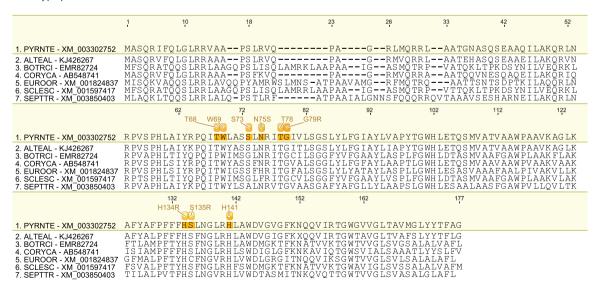


Figure 6. Amino acid sequence alignment of the SdhC family. Sequences are named by species EPPO code and NCBI gene accession number. Residues highlighted in yellow in the archetype sequence from *Pyrenophora teres* f. sp. *teres* denote amino acid substitutions associated with fungicide resistance at an orthologous position in any of the sequences. Numerical mutation labels shown above the alignment are based on the position number of the amino acid in the archetype protein.

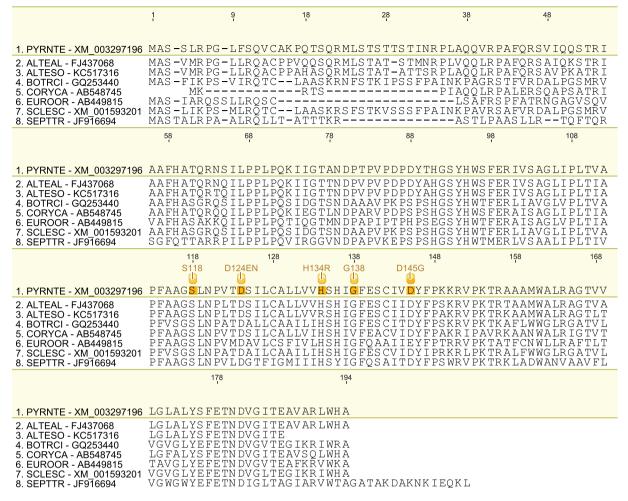


Figure 7. Amino acid sequence alignment of the SdhD family. Sequences are named by species EPPO code and NCBI gene accession number. Residues highlighted in yellow in the archetype sequence from Pyrenophora teres f. sp. teres denote amino acid substitutions associated with fungicide resistance at an orthologous position in any of the sequences. Numerical mutation labels shown above the alignment are based on the position number of the amino acid in the archetype protein.

The aim is to create a set of alignments that would be stable into the foreseeable future and would invoke the least relabelling of mutations that have already been described and published. We favour method 3 (basing the alignment on the species with the most currently described resistance mutations), but also taking into account method 1 (using the longer gene) when alternative species are candidates. We propose that Cyp51A is fitted to ASPEFU (Aspergillus fumigatus), Cyp51B and Cytb are fitted to SEPTRI (Zymoseptoria tritici), b-tubulin to ASPEND (Aspergillus nidulans), the SDH proteins to PYRNTE (Pyrenophora teres), CesA3 to PHYTIN (Phytophthora infestans) and Os-1 to BOTCIN (Botrytis cinerea). For Erg27, mutations associated with resistance have currently been described only in BOTCIN, and thus we propose this species as the archetype. The alignments have been summarised and fungicide resistance associated mutations are given in the tables. By way of example, in Cyp51B the mutation Y136F in ERYSGH would be given the label Y137F. In CANDAL (Candida albicans) the orthologous amino acid is Y132 and has been mutated to both F and H. The Y132H mutation would therefore be given the label Y137H. V151 in SEPTRI is clearly demonstrated to be orthologous to I145F in PHAKPA (Phakopsora pachyrhizi). This mutation would be labelled 1151F in PHAKPA and V151F in SEPTRI. The other proposed relabellings are listed in Tables 1 to 9.

By examining the species that have amino acid mutations with common labels, we can infer that positions 137, 148, 461, 476, 483 and 524 in Cyp51B are especially important in conferring resistance to triazole fungicides. This is consistent with numerous functional studies.<sup>10,11</sup> We expect that the alignments should assist the identification of key amino acids in target proteins of newer fungicide classes.

#### 4 THE PROPOSAL IN PRACTICE

The system must also allow for mutations to be discovered in new species. The parameters used to make the alignments are described below and can be applied to an alignment between the new species and the archetype. We envisage regularly updating the alignments based on new published knowledge.

A potential problem with the system we propose might occur if an amino acid in a newly described mutant gene corresponded to a gap in the archetype protein's sequence. In such a case, the mutation could be labelled as X50.2Y if it concerned the second extra amino acid after number 50 in the archetype sequence. To our knowledge, no examples of mutations of such poorly conserved amino acids causing resistance have been described, but the possibility remains.

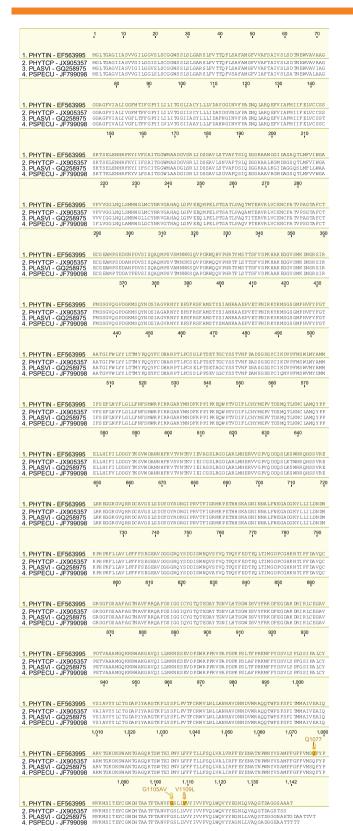
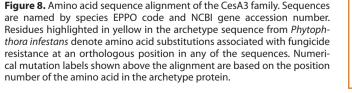


Table 10.         Abbreviations of special	ies names
Abbreviation (EPPO code)	Name of pathogen
AJELCP	Ajellomyces capsulatus
ALTEAL	Alternaria alternata
ALTEBI	Alternaria brassicicola
ALTELO	Alternaria longipes
ALTELY	Alternaria arborescens
ALTESO	Alternaria solani
ALTETO	Alternaria tomato
ASPEFL	Aspergillus flavus
ASPEFU	Aspergillus fumigatus
ASPEND	Emericella nidulans
ASPEPA BOTRCI	Aspergillus parasiticus Botryotinia fuckeliana
CANDAL	Candida albicans
СОСННЕ	Cochliobolus heterostrophus
COLLGR	Glomerella graminicola
CORYCA	Corynespora cassiicola
CERCBE	Cercospora beticola
DIDYBR	, Stagonosporopsis cucurbitacearum
ERYSGH	Blumeria graminis f. sp. hordei
ERYSGT	Blumeria graminis f. sp. tritici
EUROOR	Eurotium oryzae
FILBNF	Filobasidiella neoformans
GIBBFU	Gibberella fujikuroi
GIBBZE	Gibberella zeae
HELMSO	Helminthosporium solani
HYPMOD	Hypomyces odoratus
LEPTNO	Parastagonospora nodorum
MONGNI	Monographella nivalis
MONIFC	Monilinia fructicola
MONILA MICDMA	Monilinia laxa
MYCOFI	Microdochium majus Mycosphaerella fijiensis
MYCORA	Didymella rabiei
NEUSCR	Neurospora crassa
PENIAU	Penicillium aurantiogriseum
PENIEX	Penicillium expansum
PENIIT	Penicillium italicum
РНАКРА	Phakopsora pachyrhizi
РНҮТСР	Phytophthora capsici
PHYTDR	Phytophthora drechsleri
PHYTIN	Phytophthora infestans
PLASVI	Plasmopara viticola
PLEOAL	Pleospora allii
PODOFU	Podosphaera fusca
PODOXA	Podosphaera xanthii
PSPECU	Pseudoperonospora cubensis
PUCCRT	Puccinia triticina
PYRIOR	Magnaporthe oryzae
PYRNTE PYRNTR	Pyrenophora teres Pyrenophora tritici-repentis
PYRPBR	
RHIZSO	Pyrenopeziza brassicae Thanatephorus cucumeris
RHYNSE	Rhynchosporium secalis
SACCCE	Saccharomyces cerevisiae
SCLEHO	Sclerotinia homoeocarpa
SCLESC	Sclerotinia sclerotiorum
SEPTTR	Zymoseptoria tritici
UNCINE	Erysiphe necator
USTIMA	Ustilago maydis
VENTIN	Venturia inaequalis



	1	10	20	26	32	40	50	60
1. BOTRCI - AF435964 2. ALTEBI - AY700092 3. ALTELO - DQ887538	MAAETYS	SVSAIIRNLA SVSAIIRNLA	ROHDPTRDPS RÖHDPTRDPS	FSAQVSANG FSAQVSANG	AKTAVNAIALE AKTAANAIALE	GPESDEKTOL GPESEEKTŐL	QQELSALCS EÕELSALCS	RIDFL RIDFL
4. PLEOAL - EU711371	MAAETYS	70 70	74 74	PSAQVSANG #	99 99	100	110	120
1. BOTRCI - AF435964 2. ALTEBI - AY700092 3. ALTELO - DQ887538 4. PLEOAL - EU711371	EARAITV EHKSNHA EHKSSHA EHKSSNA	ANQQGQFPLT APNQQGQFPLT AGKSGQFPLT	LPDTPNELGA PAQEPSDEGA PAQEPPEEGA PAQEPPEEGV 40	APSAFADVLT ALYTPAGVFR LYTPAGVFR LYTQAGAIR 150	3 – – APSRASK GNNGPPAQGRF GNSGPPAQGRF I SNGPPGQARF 180	GSNKERAIWV GSNKERAIWV GSNKERATWV	NSLLAAREA SNWLAAKESI SNWLAAKESI SNWLAAKESI 180	PTGGE NGDPE NGDPE NGGPE 190
1. BOTRCI - AF435964 2. ALTEBI - AY700092 3. ALTELO - DQ887538 4. PLEOAL - EU711371	RPPKFTK QPAAA QPAAA EPAVA 20	ALTEEQLNYLR ALTEEQLNYLR ALTEEQFNYLR	EHVDHQSKQI VHLNQQADQI VHLNQQADQI VHLHQQADQI 7	RNQREHIDN RNQREHIDN RNQREHIDN	VHAQLFEQKQF LSQEVNKQLTI LSQEVNKQLTI LSQEVNKQLTI P 24	QSMVF-EHGI QSMVF-EHGI QSMVF-EHGI	EDIGALKRE EDIGALKRE EDIGALKRE	LKKHQ LGKHQ LGKHQ LGKHQ
1. BOTRCI - AF435964 2. ALTEBI - AY700092 3. ALTELO - DQ887538 4. PLEOAL - EU711371	QANEAFC QANLAFC QANLAFC QANLAFC 270	KALREIGEIV KALREIGAIV KALREIGAIV KALREIGAIV KALREIGAIV 280	FAVARGDLSK FAVAMGDLSK FAVAMGDLSK FAVAMGDLSK 290	KVQIHSVEMI KVLIHAKEMI KVLIHAKEMI KVLIHAKEMI 300	DPEITTFKRVI DPEITLFKRTI DPEITLFKRTI DPEITLFKRTI ano	NTMMDQLQIF	SSEVSRVAR.	EVGTE EVGTE EVGTE EVGTE
1. BOTRCI - AF435964 2. ALTEBI - AY700092 3. ALTELO - DQ887538 4. PLEOAL - EU711371	GRIGGOA	NLPGVAGTWA	ELTD-INGMA	KNLTDOVRE	IAVVTTAVAMG IAVVTTAVAMG IAVVTTAVAHG 380	DLSRKTERPA	RGETLOLOO	TINSM
1. BOTRCI - AF435964 2. ALTEBI - AY700092 3. ALTELO - DQ887538 4. PLEOAL - EU711371	VDQLQSF VDQLQSF	AAEVTRVARD ATQVTKVARD ATQVTKVARD ATEVTKVARD	VGTEGKLGGÇ VGTEGKLGGÇ	AEIEG <mark>VO</mark> GMU AEIAGVKGMU AEIAGVKGMU	N <mark>N</mark> TLIVNVNAM NNELTVNVNAM NNELTVNVNAM	IAQN LTTQVRD IAQN LTTQVRD	IAQVTTAVA IAQVTTAVA	QGNLT QGNLT
1. BOTRCI - AF435964 2. ALTEBI - AY700092 3. ALTELO - DQ887538 4. PLEOAL - EU711371	QKVQAEC	KGEIKQLKET KGEILELKNT KGEILELKNT KGEILELKNT 490	INSMVDQLQQ INRMVDQLQQ INRMVDQLQQ INRMVDQLQQ 800	FAREVTKIA	T447S REVGTEGRLGO	QATVHDVEGT	WRDLTENVN WKDLTENVN WKDLTENVN WKDLTENVN 540	GMAMN GMAMN GMAMN GMAMN
1. BOTRCI - AF435964 2. ALTEBI - AY700092 3. ALTELO - DQ887538 4. PLEOAL - EU711371	LTTQVRE LTTQVRE LTTQVRE LTTQVRE	IAKVTTAVAR IAEVTTAVAR IAEVTTAVAR IAEVTTAVAR 580	GDLTKKIEVE GDLSRKVKAE GDLSRKVKAE GDLSRKVKAE 570	VQGEIASLKI VQGEILSLK VQGEILSLK VQGEILSLK 580	DTINTMVDRLS ITINTMVDRLN ITINTMVDRLN ITINTMVDRLN 590	TFAFEVSKVA TFAQEVSKVA TFAQEVSKVA TFAQEVSKVA 600	REVGTDGTL REVGTDGIL REVGTDGIL REVGTDGIL	GGQAQ GGQAQ GGQAQ GGQAQ
1. BOTRCI - AF435964 2. ALTEBI - AY700092 3. ALTELO - DQ887538 4. PLEOAL - EU711371	VDNVEGK VDNVEGK VDNVEGK VDNVEGK	WKDLTENVNT WKDLTNNVNT WKDLTNNVNT WKDLTNNVNT 630	MARNLTTQVF MAQNLTLQVF MAQNLTLQVF MAQNLTLQVF 640	GISTVTQAI SISEVTQAI SISEVTQAI SISEVTQAI 690	ANGDMSQKIEV AKGDMSRRVHV AKGDMSRRVHV 860	AAAGEILILK DAEGEIRLLK DAEGEIRLLK DAEGEIRLLK 670	ETINNMVDR DTVNDMVMR DTVNDMVMR DTVNDMVMR 680	LSIFS LDEWS LDEWS LDEWS
1. BOTRCI - AF435964 2. ALTEBI - AY700092 3. ALTELO - DQ887538 4. PLEOAL - EU711371	NEVQRVA LAVKRVA LAVKRVA LAVKRVA	AKDVGVDGKMG ARDVGVDGKMG ARDVGVDGKMG ARDVGVDGKMG 700	SQADVAGIGG SQADVRDIDG SQADVRDIDG SQADVRDIDG 710	RWKEITTDVI RWKEITTDVI RWKEITTDVI RWKEITTDVI 720	NTMANNLTTQV NTMAQNLTSQV NTMAQNLTSQV NTMAQNLTSQV 730	RAFGDITNAA RAFGDITNAA RAFGDITNAA 740	TDGDFTKLI MEGKFT-QI MEGKFT-QI MEGKFT-QI 750 7750	TVEAS TVEAS TVEAS TVEAS
1. BOTRCI - AF435964 2. ALTEBI - AY700092 3. ALTELO - DQ887538 4. PLEOAL - EU711371	GEMDELK	RKINOMVSSL	RESTORNTAA	REAAELANK	FKSEFLANMSE	ETRTPMNGTT	GMTQLTLDT GMTQLTLDT	DLTHA
1. BOTRCI - AF435964 2. ALTEBI - AY700092 3. ALTELO - DQ887538 4. PLEOAL - EU711371	QREMLNI QREMLTI QREMLTI QREMLTI	VHN LAN SLLT VHN LAGQLLT VHN LAGQLLT VHN LAGQLLT 840	IIDDILDISK IIDDILDISK IIDDILDISK	IEAN RMVMEI	EIPFSMRGTIE EIPFSMRGTIE EIPFSMRGTIE	NALKSSASRA NALKSLASRA NALKSLASRA	NERKLNLAY NERKLNLAY	DVSYK DVSYK
1. BOTRCI - AF435964 2. ALTEBI - AY700092 3. ALTELO - DQ887538 4. PLEOAL - EU711371	VPDYVVG	DSFRLRQVIL DSFRLRQIIL DSFRLRQIIL DSFRLRQIIL	NLVGNAIKLT NLVGNAIKFT	EPGEVKVAI ELGEVKVAI EHGEVKVAI	SMAQEQECDPI SMAÕEÕECDPI	HYVFQFAVSD HYVFQFAVSD HYVFQFAVSD	TGIGIRGDK TGIGIRGDK TGIGIRGDK	LNLIF LNLIF
1. BOTRCI - AF435964 2. ALTEBI - AY700092 3. ALTELO - DQ887538 4. PLEOAL - EU711371	DTFQQAD DTFQQAD DTFQQAD DTFQQAD	GSMTRKFGGT GSTTRKFGGT GSTTRKFGGT GSTTRKFGGT	GLGLSISKRI GLGLSISKRI GLGLSISKRI GLGLSISKRI 1.000	VNLMRGDVW VTLMGGRMW VTLMGGRMW VTLMGGRMW	VESDAGKGSVF VESDVGKGSVF VESDFGKGSVF	YFTCTVRLAT YFTYKVRLGK YFTCKVRLGK YFTLTVRLGK 1,030	SDISFIQKQ PAIQAIQPQ PAIQAIQPQ PEITAIQPQ 1.044	LVAYK LVAYK LLHNK
1. BOTRCI - AF435964 2. ALTEBI - AY700092 3. ALTELO - DQ887538 4. PLEOAL - EU711371	GHNVLFI QHTVLFV QHTVLFV QHTVLFV 1,050	DKGQTGHGKE VDQGNTGFSDE VDQGNTGFSDQ VDQRNTGFSDQ 1,060	IITMLTQLGI ITEHLKTLDI IIEHLKTLDI IIEHLKALDI 1,070	VPVVVDSEQI VPMVVHSVEI VPMVVHSVEI VPMVVHSVEI 1,000	HTILLGNGRTK EVPETI EVPETI EVPETI 1.090	EKIASTYDVI KRTDMPYDCV KRTDMPYDCV KRADMPYDCV 1,100	VVDSIESAR IVDNDKTAR IVDNDKTAR IVDNDKTAR IVDNDKTAR 1,110	KLRSI ELRIA ELRIA ELRIA
1. BOTRCI - AF435964 2. ALTEBI - AY700092 3. ALTELO - DQ887538 4. PLEOAL - EU711371	DEFKYIF ERFKYIF ERFKYIF ERFKYIF	IVLLAPVIHV LVMLTPHVCI LVMLTPHVCI LVMLTPHVCI 1,130	SLKSALDLGI SLRSALENGI SLRSALENGI SLRSALENGI 1,140	TSYMTTPCL SSYMTTPCL SSYMTTPCL SRYMTTPCL 1,190	FIDLGNGMIPA PIDLGNALIPA PIDLGNALIPA PIDLGNALIPA 1,160	LENRAAPSLA LDGRAAPLVS LDGRAAPLVS LDGRAAPLVS 1.170	DNTKSFDIL DHSKSFQIL DHSKSFQIL DHSKSFQIL 1.180	LAEDN LAEDN LAEDN LAEDN
1. BOTRCI - AF435964 2. ALTEBI - AY700092 3. ALTELO - DQ887538 4. PLEOAL - EU711371								
1. BOTRCI - AF435964 2. ALTEBI - AY700092 3. ALTELO - DQ887538 4. PLEOAL - EU711371	IALTAHA IALTAHA IALTAHA IALTAHA 1,254	MLGDREKCIQ MVGDREKCLA MVGDREKCLA MVGDREKCLA 1,864	AQMDEYLSKF AQMDDYLSKF AQMDDYLSKF AQMDDYLSKF AQMDDYLSKF 1.274	PLKQNHLIQT PLRQNQLIQT PLRQNQLIQT PLRQNQLIQT 1,284	ILKCATLGGAI ILRCATVGSTM ILRCATVGSTM ILRCATVGSTI 1294	LEKGREVRQS YDHSSEPRYT YDHSSEPRYT YDHTSEPRYT 1.304	ANEESP ASSHLPNIT ASSHLPNIA ASSHIPKLD 1,315	NS LPGKG LPAKG MPGNS
1. BOTRCI - AF435964 2. ALTEBI - AY700092 3. ALTELO - DQ887538 4. PLEOAL - EU711371	NDNGGAS	SSSGGLPSPG	KKRPQLEARG	FTERG	G-AQSPNLLAV	DOTDNGSVER	LLLRSHSS	

**Figure 9.** Amino acid sequence alignment of the OS-1 family. Sequences are named by species EPPO code and NCBI gene accession number. Residues highlighted in yellow in the archetype sequence from *Botrytis cinerea* denote amino acid substitutions associated with fungicide resistance at an orthologous position in any of the sequences. Numerical mutation labels shown above the alignment are based on the position number of the amino acid in the archetype protein.

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We hope that future studies will refer to the archetype by indicating that the mutation X123Y in the target protein associated with resistance corresponds to the archetype *X145Y* and refer to this paper or a related web page for support.

We suggest that other target genes from medically important fungi (e.g. the FKS1/2 genes that are targets of Echinocandins) and from herbicide- and insecticide- resistant weeds and insects might also benefit from this approach.

We commend this scheme to the community and seek comment and support. And we urge journal editors to encourage authors to use this new system.

## **5 NOTE ON THE ALIGNMENTS**

Amino acid sequences were downloaded from NCBI GenBank and annotated with reported amino acid substitutions<sup>8,12–15</sup> using Geneious 6.1.8 software (Biomatters). Alignments of sequences were generated using the ClustalW<sup>16</sup> algorithm with Blosum scoring matrix, gap opening penalty 10, gap extension penalty 0.5 and free end gaps.

The alignments are available as .doc files and as fasta files in the supporting information.

## SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

### REFERENCES

- 1 Lucas JA, Hawkins NJ and Fraaije BA, The evolution of fungicide resistance. *Adv Appl Microbiol* **90**:29–92 (2015).
- 2 Grimmer MK, van den Bosch F, Powers SJ and Paveley ND, Fungicide resistance risk assessment based on traits associated with the rate of pathogen evolution. *Pest Manag Sci* **71**:207–215 (2015).
- 3 Del Sorbo G, Schoonbeek H and De Waard MA, Fungal transporters involved in efflux of natural toxic compounds and fungicides. *Fungal Genet Biol* **30**:1–15 (2000).
- 4 Cools HJ, Fraaije BA, Bean TP, Antoniw J and Lucas JA, Transcriptome profiling of the response of *Mycosphaerella graminicola* isolates to an

azole fungicide using cDNA microarrays. *Mol Plant Pathol* 8:639–651 (2007).

- 5 Oliver RP and Hewitt HG, *Fungicides in Crop Protection*, 2nd edition. CABI Publishing, Wallingford, Oxon, UK (2014).
- 6 Sierotzki H, Parisi S, Steinfeld U, Tenzer I, Poirey S and Gisi U, Mode of resistance to respiration inhibitors at the cytochrome bc<sub>1</sub> enzyme complex of *Mycosphaerella fijiensis* field isolates. *Pest Manag Sci* 56:833–841 (2000).
- 7 FRAC Code List. [Online]. FRAC. Available: http://www.frac.info/docs/ default-source/publications/pathogen-risk/pathogen-risk-list.pdf? sfvrsn=8 [10 January 2016].
- 8 Ishii H and Hollomon DW, Fungicide Resistance in Plant Pathogens: Principles and a Guide to Practical Management. Springer, Tokyo, Japan (2015).
- 9 *EPPO Global Database*. [Online]. EPPO. Available: https://gd.eppo.int [21 January 2016].
- 10 Kelly HM, Wright DL, Dufault NS and Marois JJ, Decision models for fungicide applications for soybean rust. *Plant Hlth Prog* 16:80-83 (2015).
- 11 Cools HJ, Parker JE, Kelly DE, Lucas JA, Fraaije BA and Kelly SL, Heterologous expression of mutated eburicol 14α-demethylase (CYP51) proteins of *Mycosphaerella graminicola* to assess effects on azole fungicide sensitivity and intrinsic protein function. *Appl Environ Microbiol* **76**:2866–2872 (2010).
- 12 Becher R and Wirsel SG, Fungal cytochrome P450 sterol 14α-demethylase (CYP51) and azole resistance in plant and human pathogens. *Appl Microbiol Biotechnol* **95**:825–840 (2012).
- 13 Morio F, Loge C, Besse B, Hennequin C and Le Pape P, Screening for amino acid substitutions in the *Candida albicans* Erg11 protein of azole-susceptible and azole-resistant clinical isolates: new substitutions and a review of the literature. *Diagn Microbiol Infect Dis* **66**:373–384 (2010).
- 14 Howard SJ and Arendrup MC, Acquired antifungal drug resistance in Aspergillus fumigatus: epidemiology and detection. Med Mycol 49(Suppl 1):S90–S95 (2011).
- 15 Sagatova AA, Keniya MV, Wilson RK, Monk BC and Tyndall JD, Structural insights into binding of the antifungal drug fluconazole to Saccharomyces cerevisiae lanosterol 14α-demethylase. Antimicrob Agents Chemother 59:4982–4989 (2015).
- 16 Thompson JD, Higgins DG and Gibson TJ, CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucl Acids Res 22:4673–4680 (1994).