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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 current 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.

Background

Evolved resistance to fungicides is a major problem limiting our ability to control agricultural, medical and veterinary pathogens^{1, 2}. 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 pumps³, or to over-expression of target genes⁴ 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 (see⁵ box 6.1 page 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 G143A⁶. Further alterations can be amino acid deletions designated with a Δ and insertions with an “ins”.

Target site amino acid substitutions have been described for seven fungicide groups (named here according to the FRAC convention,⁷) and their target proteins. These are C3 and cytochrome b (Cytb) (Table 1); G1 and two sterol C14-demethylases (paralogs Cyp51A and Cyp51B), (Tables 2 and 3); B1/B2 and *b*-tubulin, (Table 4); C2 and three of the sub-units of the succinate dehydrogenase complex (SdhB, SdhC and SdhD) (Table 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 NIK1⁸)(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 132 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 creates unnecessary confusion and obscures 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.

The Proposal

The differences in numbering arise from the different lengths of the fungicide target protein in each species. The purpose of the current 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 refers to the orthology between proteins from different species, from ‘amino 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.

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 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 to functionally characterise mutations.

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 (Figures 1 to 9) and tables of putatively orthologous amino acids in other species where fungicide resistance has been reported (Tables 1 to 9). The species included in these tables and alignments have been referred to by their European and Mediterranean Plant Protection Organization (EPPO) codes⁹ as listed in Table 10.

The alignments for *b*-tubulin and Cytb are essentially co-linear in fungi studied to date and hence there are no changes to be made on 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 the fungicide resistance concerned.
4. Fitted to the gene from the species that was the first species for the fungicide resistance concerned.

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 I151F in PHAKPA and V151F in SEPTRI. The other proposed relabellings are listed in Tables 1 to 9.

By examining the species that have amino acids mutations with common labels, we can infer that positions 137, 148, 461, 467, 483 and 524 in Cyp51B are especially important in conferring resistance to triazole fungicides. This is consistent with numerous functional studies^{10, 11}. We expect that the alignments should assist the identification of key amino acids in target proteins of newer fungicide classes.

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.

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.

Note on the alignments

Amino acid sequences were downloaded from NCBI GenBank and annotated with reported amino acid substitutions^{8, 12-15}, using Geneious 6.1.8 software (Biomatters). Alignments of sequences were generated using ClustalW¹⁶ algorithm with Blosom scoring matrix, gap opening penalty 10, gap extension penalty 0.5, free end gaps.

The alignments are available as .doc file and as fasta files as supplementary data,

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Figure Legends

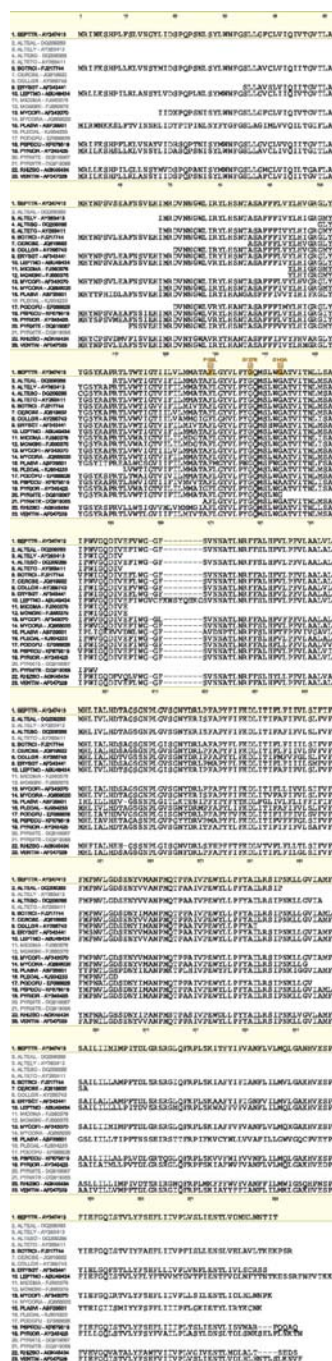


Figure 1. Amino acid sequence alignment of the Cytb family. Sequences are named by species EPPO code and NCBI gene accession number. Residues highlighted in yellow in the archetype sequence from *Zymoseptoria tritici* denote amino acid substitutions associated with fungicide resistance at an orthologous 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.

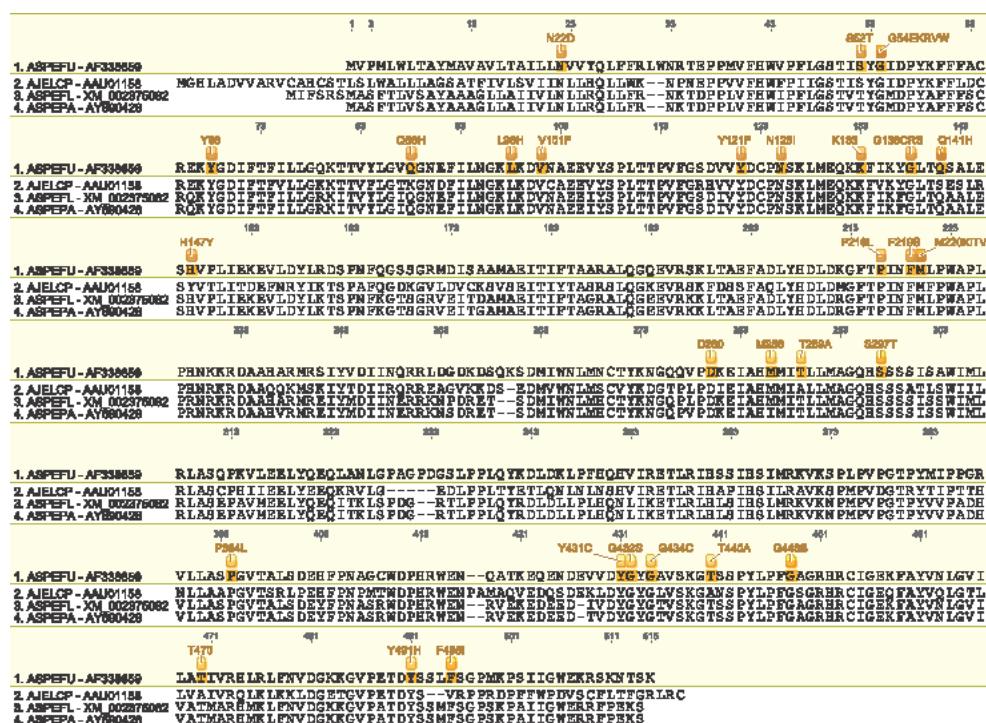


Figure 2. Amino acid sequence alignment of the Cyp51A family. Sequences are named by species EPP0 code and NCBI gene accession number. Residues highlighted in yellow in the archetype sequence from *Aspergillus fumigatus* (Cyp51A) denote amino acid substitutions associated with fungicide resistance at 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.

1. SEPTTR - AY253234	MGLLQEVLAQFDAQFG-----QTHLNKLVLGLGFLAFSTLAILNLVLSQLLF
2. ASPEFL - KOC13903	MGILAVILDSVCERCS-----GSSLNMLSTVALLSILVSVVINVLRQLLF
3. CANDAL - AF163660	MAIVETVID-----GINYFLSLSVTQOISILLGVPFVYNLVWQILY
4. CERCBE - HM778021	MGLLQDVAVAFDNHFG-----QTATNKLVLASTFFLLSVVNVLRQVLF
5. ERYSGH - AJ578781	MGISLSFMFYLOPDL-----QLGFGIALASGILSILLLLTFFINVLKQLLF
6. ERYSGT - AJ578781	MNGKPEFMSFYLOPDL-----QCGFSIALASGILSILLLLTFFINVLKQLLF
7. FILBNF - AF228914	MSAIIQVQVQLLQVQAQFPPWFALFESLKVAVAVGIPALLTIGINVFQQLCF
8. MONIFC - AF470821	MGVLETLAAGVAAEIS-----QRSIGTIAAGVAAEVVLAUVINVLQVLF
9. MYCOFI - XP_007928762	MGLLQDADLAEFAQFG-----STATNKLVLAFESIEFAVSVLVNLVQLLF
10. PHAKPA - KC741476	MSSSV---LIDQY-----SFTSLTSLTSLTSLTIIIVLVNLQDLF
11. PUCCRT - FJ978883	MSVIGSLLEPIG-----SFTFNOVLTIVLVAVSVIISINIFDQLAI
12. SACDCE - AAB88433	MSATKSIVGEALRYVNI-----GLSHFLALFLAQRISLIIPFTYNIVWQLLY
13. UNCINE - AAC48812	MYIADILSLLTQQT-----RYGWIFMVTSIAFSIILLAVGLNVLSQLLF
1. SEPTTR - AY253234	RGRSSDPPLVFWVFPFISLTITYGIDPYKFFFSCKEYGVDTFTFILLGKKTIVCLGT
2. ASPEFL - KOC13903	RNY--KEPPVFWHFWPFIISTISYGMDFRFFNCRKRYGDTFTFVLLGKKTIVYLG
3. CANDAL - AF163660	SIRKDRAPLWFWYWPFGSAAISYGCQPYEFESCRQYGVDTFTFILLGKKTIVYLG
4. CERCBE - HM778021	RQPKHEPPVFWHFWPFIISTISYGIDPYKFFFSCKEYGVDTFTFILLGKKTIVCLGT
5. ERYSGH - AJ578781	RNP--HEPPVFWHFWPFIISTISYGMDFRFFNCRKRYGDTFTFILLGKKTIVYLG
6. ERYSGT - AJ578781	RNP--HEPPVFWHFWPFIISTISYGMDFRFFNCRKRYGDTFTFILLGKKTIVYLG
7. FILBNF - AF228914	PRKDLPPVFWHFWPFIISTISYGMDFRFFNCRKRYGDTFTFILLGKKTIVYLG
8. MONIFC - AF470821	ANP--HEPPVFWHFWPFIISTISYGIDPYKFFFSCKEYGVDTFTFILLGKKTIVYLG
9. MYCOFI - XP_007928762	RNP--HEPPVFWHFWPFIISTISYGIDPYKFFFSCKEYGVDTFTFILLGKKTIVYLG
10. PHAKPA - KC741476	-KDRHTPPLVFWHFWPFIISTISYGMDFRFFNCRKRYGDTFTFILLGKKTIVYLG
11. PUCCRT - FJ978883	PKDPTAPPVFWHFWPFIISTISYGMDFRFFNCRKRYGDTFTFILLGKKTIVYLG
12. SACDCE - AAB88433	SIRKDRAPLWFWYWPFGSAAISYGCQPYEFESCKEYGVDTFTFILLGKKTIVYLG
13. UNCINE - AAC48812	RNP--HEPPVFWHFWPFIISTISYGIDPYKFFFSCKEYGVDTFTFILLGKKTIVYLG
1. SEPTTR - AY253234	KGNDFILNGKILKDVNAEYISPLTTFVFGKDVVYDCPNKLMQKKEVRYGLTTHAL
2. ASPEFL - KOC13903	KGNDFILNGKILKDVNAEYISPLTTFVFGKDVVYDCPNKLMQKKEVRYGLTTHAL
3. CANDAL - AF163660	KGEFEVFNALISDVSAEDAYKHLTTFVFGKDVVYDCPNKLMQKKEVRYGLTTHAL
4. CERCBE - HM778021	KGNDFILNGKILKDVNAEYISPLTTFVFGKDVVYDCPNKLMQKKEVRYGLTTHAL
5. ERYSGH - AJ578781	QGNDFILNGKILKDVNAEYISPLTTFVFGKDVVYDCPNKLMQKKEVRYGLTTHAL
6. ERYSGT - AJ578781	QGNDFILNGKILKDVNAEYISPLTTFVFGKDVVYDCPNKLMQKKEVRYGLTTHAL
7. FILBNF - AF228914	KGNDFILNGKILKDVNAEYISPLTTFVFGKDVVYDCPNKLMQKKEVRYGLTTHAL
8. MONIFC - AF470821	KGNDFILNGKILKDVNAEYISPLTTFVFGKDVVYDCPNKLMQKKEVRYGLTTHAL
9. MYCOFI - XP_007928762	KGNDFILNGKILKDVNAEYISPLTTFVFGKDVVYDCPNKLMQKKEVRYGLTTHAL
10. PHAKPA - KC741476	EGNTLVNLGKILSEVNAEYISPLTTFVFGKDVVYDCPNKLMQKKEVRYGLTTHAL
11. PUCCRT - FJ978883	EGNTLVNLGKILSEVNAEYISPLTTFVFGKDVVYDCPNKLMQKKEVRYGLTTHAL
12. SACDCE - AAB88433	KGEFEVFNALISDVSAEDAYKHLTTFVFGKDVVYDCPNKLMQKKEVRYGLTTHAL
13. UNCINE - AAC48812	QGNDFILNGKILKDVNAEYISPLTTFVFGKDVVYDCPNKLMQKKEVRYGLTTHAL
1. SEPTTR - AY253234	QSYVTLIAETRTQFDRNPEKRFASISGTLDEPPLAELTIYTASRLQGEVREG
2. ASPEFL - KOC13903	RSYVRLITEVEDEVOKSS---ALQCPNGVFDVCKTIAETIYTASRLQGEVREG
3. CANDAL - AF163660	KRYVFKIRZYLNYFTDSEKLEKETHGVANVMKTOEITITFASRLQGEVREG
4. CERCBE - HM778021	QSYVTLITEETROFSEKNSPEKRFASISGTLDEPPLAELTIYTASRLQGEVREG
5. ERYSGH - AJ578781	RSYVPIQHEVKSFTIEKCD---DFRESKINTIDAVNAETIYTASRLQGEVREG
6. ERYSGT - AJ578781	RSYVPIQHEVKSFTIEKCD---DFRESKINTIDAVNAETIYTASRLQGEVREG
7. FILBNF - AF228914	RSYVPIQHEVKSFTIEKCD---DFRESKINTIDAVNAETIYTASRLQGEVREG
8. MONIFC - AF470821	RSYVPIQHEVKSFTIEKCD---DFRESKINTIDAVNAETIYTASRLQGEVREG
9. MYCOFI - XP_007928762	RSYVPIQHEVKSFTIEKCD---DFRESKINTIDAVNAETIYTASRLQGEVREG
10. PHAKPA - KC741476	RSYVPIQHEVKSFTIEKCD---DFRESKINTIDAVNAETIYTASRLQGEVREG
11. PUCCRT - FJ978883	RSYVPIQHEVKSFTIEKCD---DFRESKINTIDAVNAETIYTASRLQGEVREG
12. SACDCE - AAB88433	RSYVPIQHEVKSFTIEKCD---DFRESKINTIDAVNAETIYTASRLQGEVREG
13. UNCINE - AAC48812	RSYVPIQHEVKSFTIEKCD---DFRESKINTIDAVNAETIYTASRLQGEVREG
1. SEPTTR - AY253234	FDSFADLYLHLDLMDGFTPINFMLFWAPLPQNRDRDYAQKKMSETYMSIIQKRRES--
2. ASPEFL - KOC13903	FDSFADLYLHLDLMDGFTPINFMLFWAPLPQNRDRDYAQKKMSETYMSIIQKRRES--
3. CANDAL - AF163660	FDSFADLYLHLDLMDGFTPINFMLFWAPLPQNRDRDYAQKKMSETYMSIIQKRRES--
4. CERCBE - HM778021	FDSFADLYLHLDLMDGFTPINFMLFWAPLPQNRDRDYAQKKMSETYMSIIQKRRES--
5. ERYSGH - AJ578781	FDSFADLYLHLDLMDGFTPINFMLFWAPLPQNRDRDYAQKKMSETYMSIIQKRRES--
6. ERYSGT - AJ578781	FDSFADLYLHLDLMDGFTPINFMLFWAPLPQNRDRDYAQKKMSETYMSIIQKRRES--
7. FILBNF - AF228914	FDSFADLYLHLDLMDGFTPINFMLFWAPLPQNRDRDYAQKKMSETYMSIIQKRRES--
8. MONIFC - AF470821	FDSFADLYLHLDLMDGFTPINFMLFWAPLPQNRDRDYAQKKMSETYMSIIQKRRES--
9. MYCOFI - XP_007928762	FDSFADLYLHLDLMDGFTPINFMLFWAPLPQNRDRDYAQKKMSETYMSIIQKRRES--
10. PHAKPA - KC741476	FDSFADLYLHLDLMDGFTPINFMLFWAPLPQNRDRDYAQKKMSETYMSIIQKRRES--
11. PUCCRT - FJ978883	FDSFADLYLHLDLMDGFTPINFMLFWAPLPQNRDRDYAQKKMSETYMSIIQKRRES--
12. SACDCE - AAB88433	FDSFADLYLHLDLMDGFTPINFMLFWAPLPQNRDRDYAQKKMSETYMSIIQKRRES--
13. UNCINE - AAC48812	FDSFADLYLHLDLMDGFTPINFMLFWAPLPQNRDRDYAQKKMSETYMSIIQKRRES--
1. SEPTTR - AY253234	-KTGEEEDMIENLMQ--CKYKDGNAIPDRETAENMIALLMAGCHSSSATESWITLRL
2. ASPEFL - KOC13903	--SKKDS EOMVNNLMS--CMYKDGTPVPDRETAENMIALLMAGCHSSSATESWITLRL
3. CANDAL - AF163660	--DIDFNRDLIDSLLHSTYKDGVRMTDQETANLLIGILMGGCHTSASTANWFLLEL
4. CERCBE - HM778021	KTS EHGCEEDMIENLMQ--CTYKDGTPVPDRETAENMIALLMAGCHSSSATESWITLRL
5. ERYSGH - AJ578781	-ETDSDNLODMNQLMR--SSYKDGTPVPDRETAENMIALLMAGCHSSSATESWITLRL
6. ERYSGT - AJ578781	-ETDSDNLODMNQLMR--SSYKDGTPVPDRETAENMIALLMAGCHSSSATESWITLRL
7. FILBNF - AF228914	--SDEE--DMTENLQS--CKYRNGVPLSDRDIAEMIALLMAGCHTSATSTWITLRL
8. MONIFC - AF470821	-TEAFKS DTMNQLMR--SSYKDGTPVPDRETAENMIALLMAGCHSSSATESWITLRL
9. MYCOFI - XP_007928762	PTSGKKEQDMTENLQS--CKYRNGVPLSDRDIAEMIALLMAGCHTSATSTWITLRL
10. PHAKPA - KC741476	--RQDFENDMLEVLQ--QYKDGRLSDRETAEMIALLMAGCHTSATSTWITLRL
11. PUCCRT - FJ978883	--REGQLGDMIDSLOQ--QYKDGRLSDRETAEMIALLMAGCHTSATSTWITLRL
12. SACDCE - AAB88433	--DIQ--DRDLIDSLEMSYKDGVRMTDQETANLLIGILMGGCHTSASTANWFLLEL
13. UNCINE - AAC48812	-RSGENKHDIMNQLMR--SYKDGTPVPDRETAENMIALLMAGCHSSSATESWITLRL
1. SEPTTR - AY253234	AARPDITQDELLQEQKDMGLGVNADGS--IKELTYANLSKLTLLNQVVKETLRIBAPV
2. ASPEFL - KOC13903	AASPEITEELYEQEQLRILGHQ----MPPELTENLQKLDLMAKYIKETLRIBAPV
3. CANDAL - AF163660	GEKPHLDQVLYEQEVVLELKEG----GDLNLDITYEDLQKLPVNNNTIKETLRIBAPV
4. CERCBE - HM778021	ATRRDIQDELYEQEQLRILGHQ----IKELTYANLSKLTLLNQVVKETLRIBAPV
5. ERYSGH - AJ578781	AARPDIITEELYEQEQLRILGHQ----LPPELYEDLSKLSLHQNVLKEVLRIBAPV
6. ERYSGT - AJ578781	AARPDIITEELYEQEQLRILGHQ----LPPELYEDLSKLSLHQNVLKEVLRIBAPV
7. FILBNF - AF228914	ADRPDVVEALYEQEQKDLGNPDGTF--DYRYEDLKEDELIMDSIIRETLRIBAPV
8. MONIFC - AF470821	AARPDIMEELYEQEQLRILGHQ----LPPELYEDLSKLSLHQNVLKEVLRIBAPV
9. MYCOFI - XP_007928762	AARPDITQDELLQEQKDMGLGVNADGS--IKELTYANLSKLTLLNQVVKETLRIBAPV
10. PHAKPA - KC741476	ASCPDLVDRILRREQEVEFGKDGSG--ELEDLDYDRLOTPPLNLSCKEVLRLBPPV
11. PUCCRT - FJ978883	ASRPDIIVAEILRREQEIDLFGKPGQTDQELDPLDLERVSPLMIACKEVLRIBPPV
12. SACDCE - AAB88433	AERPVOQELYEQEQLRILGHQ----GGKKELTYDLEQLMPLNQTIKETLRIBAPV
13. UNCINE - AAC48812	AARPDIMEELYEQEQLRILGHQ----PPPELYEDLSKLSLHQNVLKEVLRIBAPV
1. SEPTTR - AY253234	SILRVKS EPIEG-----TAYVIPTHTETLLAPGTTSRNDEHFPDCLHWEPEP
2. ASPEFL - KOC13903	SIRAVKN PMPVEG-----TPYVIPTSENVLS PGVTARSEHFPDCLHWEPEP
3. CANDAL - AF163660	SIRKVTN PIRIPE-----TNYIVRGEYVLVS PGYATTSERYVTDNEDFPDPR

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.

	1	10	20	30	40	50
		H4LY				Y62NS
1. ASPEND berA beta-tubulin - M17519	MREIVHLATGQCQNGTGAAPFQMTISGHEGLDGSQVYNGTSDLCLERMNVTFNEASGNKTVPRAV					
2. BOTRC1 - Z88283	MREIVHLATGQCQNGTGAAPFQMTISGHEGLDGSQVYNGTSDLCLERMNVTFNEASGNKTVPRAV					
3. CERC8E - AY585373	MREIVHLATGQCQNGTGAAPFQMTISGHEGLDGSQVYNGTSDLCLERMNVTFNEASGNKTVPRAV					
4. COCHIE - AB000971	MREIVHLATGQCQNGTGAAPFQMTISGHEGLDGSQVYNGTSDLCLERMNVTFNEASGNKTVPRAV					
5. GIBBFU beta 1-tubulin - U27303	MREIVHLATGQCQNGTGAAPFQMTISGHEGLDGSQVYNGTSDLCLERMNVTFNEASGNKTVPRAV					
6. GIBBFU beta 2-tubulin - KF771181	MREIVHLATGQCQNGTGAAPFQMTISGHEGLDGSQVYNGTSDLCLERMNVTFNEASGNKTVPRAV					
7. GIBBZE beta 2-tubulin - FJ214883	MREIVHLATGQCQNGTGAAPFQMTISGHEGLDGSQVYNGTSDLCLERMNVTFNEASGNKTVPRAV					
8. HELMSO - Y10870	MREIVHLATGQCQNGTGAAPFQMTISGHEGLDGSQVYNGTSDLCLERMNVTFNEASGNKTVPRAV					
9. HYFMOD - Y12236	MREIVHLATGQCQNGTGAAPFQMTISGHEGLDGSQVYNGTSDLCLERMNVTFNEASGNKTVPRAV					
10. LEFTNO - S68822	MREIVHLATGQCQNGTGAAPFQMTISGHEGLDGSQVYNGTSDLCLERMNVTFNEASGNKTVPRAV					
11. MONFC - AY283676	MREIVHLATGQCQNGTGAAPFQMTISGHEGLDGSQVYNGTSDLCLERMNVTFNEASGNKTVPRAV					
12. MONLA - AY349149	MREIVHLATGQCQNGTGAAPFQMTISGHEGLDGSQVYNGTSDLCLERMNVTFNEASGNKTVPRAV					
13. NEUSCR - M13630	MREIVHLATGQCQNGTGAAPFQMTISGHEGLDGSQVYNGTSDLCLERMNVTFNEASGNKTVPRAV					
14. PENIAU - JN112033	MREIVHLATGQCQNGTGAAPFQMTISGHEGLDGSQVYNGTSDLCLERMNVTFNEASGNKTVPRAV					
15. PENEX - KGO42886	MREIVHLATGQCQNGTGAAPFQMTISGHEGLDGSQVYNGTSDLCLERMNVTFNEASGNKTVPRAV					
16. PENIT - KGO72779	MREIVHLATGQCQNGTGAAPFQMTISGHEGLDGSQVYNGTSDLCLERMNVTFNEASGNKTVPRAV					
17. PYRPR - KC342227	MREIVHLATGQCQNGTGAAPFQMTISGHEGLDGSQVYNGTSDLCLERMNVTFNEASGNKTVPRAV					
18. RHYNSE - X21046	MREIVHLATGQCQNGTGAAPFQMTISGHEGLDGSQVYNGTSDLCLERMNVTFNEASGNKTVPRAV					
19. SCLEHO - KF765483	MREIVHLATGQCQNGTGAAPFQMTISGHEGLDGSQVYNGTSDLCLERMNVTFNEASGNKTVPRAV					
20. SCLESC - XM 00184794	MREIVHLATGQCQNGTGAAPFQMTISGHEGLDGSQVYNGTSDLCLERMNVTFNEASGNKTVPRAV					
21. VENTIN - M87961	MREIVHLATGQCQNGTGAAPFQMTISGHEGLDGSQVYNGTSDLCLERMNVTFNEASGNKTVPRAV					

	70	80	90	100	110	120
		M73				
1. ASPEND berA beta-tubulin - M17519	LVLDLEPGTMDAVRAGPFGQLERPDMFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD					
2. BOTRC1 - Z88283	LVLDLEPGTMDAVRAGPFGQLERPDMFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD					
3. CERC8E - AY585373	LVLDLEPGTMDAVRAGPFGQLERPDMFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD					
4. COCHIE - AB000971	LVLDLEPGTMDAVRAGPFGQLERPDMFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD					
5. GIBBFU beta 1-tubulin - U27303	LVLDLEPGTMDAVRAGPFGQLERPDMFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD					
6. GIBBFU beta 2-tubulin - KF771181	LVLDLEPGTMDAVRAGPFGQLERPDMFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD					
7. GIBBZE beta 2-tubulin - FJ214883	LVLDLEPGTMDAVRAGPFGQLERPDMFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD					
8. HELMSO - Y10870	LVLDLEPGTMDAVRAGPFGQLERPDMFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD					
9. HYFMOD - Y12236	LVLDLEPGTMDAVRAGPFGQLERPDMFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD					
10. LEFTNO - S68822	LVLDLEPGTMDAVRAGPFGQLERPDMFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD					
11. MONFC - AY283676	LVLDLEPGTMDAVRAGPFGQLERPDMFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD					
12. MONLA - AY349149	LVLDLEPGTMDAVRAGPFGQLERPDMFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD					
13. NEUSCR - M13630	LVLDLEPGTMDAVRAGPFGQLERPDMFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD					
14. PENIAU - JN112033	LVLDLEPGTMDAVRAGPFGQLERPDMFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD					
15. PENEX - KGO42886	LVLDLEPGTMDAVRAGPFGQLERPDMFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD					
16. PENIT - KGO72779	LVLDLEPGTMDAVRAGPFGQLERPDMFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD					
17. PYRPR - KC342227	LVLDLEPGTMDAVRAGPFGQLERPDMFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD					
18. RHYNSE - X21046	LVLDLEPGTMDAVRAGPFGQLERPDMFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD					
19. SCLEHO - KF765483	LVLDLEPGTMDAVRAGPFGQLERPDMFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD					
20. SCLESC - XM 00184794	LVLDLEPGTMDAVRAGPFGQLERPDMFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD					
21. VENTIN - M87961	LVLDLEPGTMDAVRAGPFGQLERPDMFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD					

	130	140	150	160	170	180	190
		Q134K		A165V	F167		
1. ASPEND berA beta-tubulin - M17519	CLQGGQITBSLGGGTGAGNTLLISKREIIFOROMATISVVSFVSDTVTFPYMATISVBQL						
2. BOTRC1 - Z88283	CLQGGQITBSLGGGTGAGNTLLISKREIIFOROMATISVVSFVSDTVTFPYMATISVBQL						
3. CERC8E - AY585373	CLQGGQITBSLGGGTGAGNTLLISKREIIFOROMATISVVSFVSDTVTFPYMATISVBQL						
4. COCHIE - AB000971	CLQGGQITBSLGGGTGAGNTLLISKREIIFOROMATISVVSFVSDTVTFPYMATISVBQL						
5. GIBBFU beta 1-tubulin - U27303	CLQGGQITBSLGGGTGAGNTLLISKREIIFOROMATISVVSFVSDTVTFPYMATISVBQL						
6. GIBBFU beta 2-tubulin - KF771181	CLQGGQITBSLGGGTGAGNTLLISKREIIFOROMATISVVSFVSDTVTFPYMATISVBQL						
7. GIBBZE beta 2-tubulin - FJ214883	CLQGGQITBSLGGGTGAGNTLLISKREIIFOROMATISVVSFVSDTVTFPYMATISVBQL						
8. HELMSO - Y10870	CLQGGQITBSLGGGTGAGNTLLISKREIIFOROMATISVVSFVSDTVTFPYMATISVBQL						
9. HYFMOD - Y12236	CLQGGQITBSLGGGTGAGNTLLISKREIIFOROMATISVVSFVSDTVTFPYMATISVBQL						
10. LEFTNO - S68822	CLQGGQITBSLGGGTGAGNTLLISKREIIFOROMATISVVSFVSDTVTFPYMATISVBQL						
11. MONFC - AY283676	CLQGGQITBSLGGGTGAGNTLLISKREIIFOROMATISVVSFVSDTVTFPYMATISVBQL						
12. MONLA - AY349149	CLQGGQITBSLGGGTGAGNTLLISKREIIFOROMATISVVSFVSDTVTFPYMATISVBQL						
13. NEUSCR - M13630	CLQGGQITBSLGGGTGAGNTLLISKREIIFOROMATISVVSFVSDTVTFPYMATISVBQL						
14. PENIAU - JN112033	CLQGGQITBSLGGGTGAGNTLLISKREIIFOROMATISVVSFVSDTVTFPYMATISVBQL						
15. PENEX - KGO42886	CLQGGQITBSLGGGTGAGNTLLISKREIIFOROMATISVVSFVSDTVTFPYMATISVBQL						
16. PENIT - KGO72779	CLQGGQITBSLGGGTGAGNTLLISKREIIFOROMATISVVSFVSDTVTFPYMATISVBQL						
17. PYRPR - KC342227	CLQGGQITBSLGGGTGAGNTLLISKREIIFOROMATISVVSFVSDTVTFPYMATISVBQL						
18. RHYNSE - X21046	CLQGGQITBSLGGGTGAGNTLLISKREIIFOROMATISVVSFVSDTVTFPYMATISVBQL						
19. SCLEHO - KF765483	CLQGGQITBSLGGGTGAGNTLLISKREIIFOROMATISVVSFVSDTVTFPYMATISVBQL						
20. SCLESC - XM 00184794	CLQGGQITBSLGGGTGAGNTLLISKREIIFOROMATISVVSFVSDTVTFPYMATISVBQL						
21. VENTIN - M87961	CLQGGQITBSLGGGTGAGNTLLISKREIIFOROMATISVVSFVSDTVTFPYMATISVBQL						

	200	210	220	230	240	250
	E190DK	F207Y			L240	
1. ASPEND berA beta-tubulin - M17519	VENSDETFICINEALYDICKRKLKSNPSTGILNHLVSAVMSGVTTCLAFPGQLNSDLKHLAVN					
2. BOTRC1 - Z88283	VENSDETFICINEALYDICKRKLKSNPSTGILNHLVSAVMSGVTTCLAFPGQLNSDLKHLAVN					
3. CERC8E - AY585373	VENSDETFICINEALYDICKRKLKSNPSTGILNHLVSAVMSGVTTCLAFPGQLNSDLKHLAVN					
4. COCHIE - AB000971	VENSDETFICINEALYDICKRKLKSNPSTGILNHLVSAVMSGVTTCLAFPGQLNSDLKHLAVN					
5. GIBBFU beta 1-tubulin - U27303	VENSDETFICINEALYDICKRKLKSNPSTGILNHLVSAVMSGVTTCLAFPGQLNSDLKHLAVN					
6. GIBBFU beta 2-tubulin - KF771181	VENSDETFICINEALYDICKRKLKSNPSTGILNHLVSAVMSGVTTCLAFPGQLNSDLKHLAVN					
7. GIBBZE beta 2-tubulin - FJ214883	VENSDETFICINEALYDICKRKLKSNPSTGILNHLVSAVMSGVTTCLAFPGQLNSDLKHLAVN					
8. HELMSO - Y10870	VENSDETFICINEALYDICKRKLKSNPSTGILNHLVSAVMSGVTTCLAFPGQLNSDLKHLAVN					
9. HYFMOD - Y12236	VENSDETFICINEALYDICKRKLKSNPSTGILNHLVSAVMSGVTTCLAFPGQLNSDLKHLAVN					
10. LEFTNO - S68822	VENSDETFICINEALYDICKRKLKSNPSTGILNHLVSAVMSGVTTCLAFPGQLNSDLKHLAVN					
11. MONFC - AY283676	VENSDETFICINEALYDICKRKLKSNPSTGILNHLVSAVMSGVTTCLAFPGQLNSDLKHLAVN					
12. MONLA - AY349149	VENSDETFICINEALYDICKRKLKSNPSTGILNHLVSAVMSGVTTCLAFPGQLNSDLKHLAVN					
13. NEUSCR - M13630	VENSDETFICINEALYDICKRKLKSNPSTGILNHLVSAVMSGVTTCLAFPGQLNSDLKHLAVN					
14. PENIAU - JN112033	VENSDETFICINEALYDICKRKLKSNPSTGILNHLVSAVMSGVTTCLAFPGQLNSDLKHLAVN					
15. PENEX - KGO42886	VENSDETFICINEALYDICKRKLKSNPSTGILNHLVSAVMSGVTTCLAFPGQLNSDLKHLAVN					
16. PENIT - KGO72779	VENSDETFICINEALYDICKRKLKSNPSTGILNHLVSAVMSGVTTCLAFPGQLNSDLKHLAVN					
17. PYRPR - KC342227	VENSDETFICINEALYDICKRKLKSNPSTGILNHLVSAVMSGVTTCLAFPGQLNSDLKHLAVN					
18. RHYNSE - X21046	VENSDETFICINEALYDICKRKLKSNPSTGILNHLVSAVMSGVTTCLAFPGQLNSDLKHLAVN					
19. SCLEHO - KF765483	VENSDETFICINEALYDICKRKLKSNPSTGILNHLVSAVMSGVTTCLAFPGQLNSDLKHLAVN					
20. SCLESC - XM 00184794	VENSDETFICINEALYDICKRKLKSNPSTGILNHLVSAVMSGVTTCLAFPGQLNSDLKHLAVN					
21. VENTIN - M87961	VENSDETFICINEALYDICKRKLKSNPSTGILNHLVSAVMSGVTTCLAFPGQLNSDLKHLAVN					

	260	270	280	290	300	310
	M857L					
1. ASPEND berA beta-tubulin - M17519	MVFPERLEITWVGFAITSGAGSFRAVSVPELTQOMDKNMAASDFRNGRYLTCCHAYFRGE					
2. BOTRC1 - Z88283	MVFPERLEITWVGFAITSGAGSFRAVSVPELTQOMDKNMAASDFRNGRYLTCCHAYFRGE					
3. CERC8E - AY585373	MVFPERLEITWVGFAITSGAGSFRAVSVPELTQOMDKNMAASDFRNGRYLTCCHAYFRGE					
4. COCHIE - AB000971	MVFPERLEITWVGFAITSGAGSFRAVSVPELTQOMDKNMAASDFRNGRYLTCCHAYFRGE					
5. GIBBFU beta 1-tubulin - U27303	MVFPERLEITWVGFAITSGAGSFRAVSVPELTQOMDKNMAASDFRNGRYLTCCHAYFRGE					
6. GIBBFU beta 2-tubulin - KF771181	MVFPERLEITWVGFAITSGAGSFRAVSVPELTQOMDKNMAASDFRNGRYLTCCHAYFRGE					
7. GIBBZE beta 2-tubulin - FJ214883	MVFPERLEITWVGFAITSGAGSFRAVSVPELTQOMDKNMAASDFRNGRYLTCCHAYFRGE					
8. HELMSO - Y10870	MVFPERLEITWVGFAITSGAGSFRAVSVPELTQOMDKNMAASDFRNGRYLTCCHAYFRGE					

Figure 4. Amino acid sequence alignment of the *b*-tubulin family. Sequences are named by species EPP0 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.

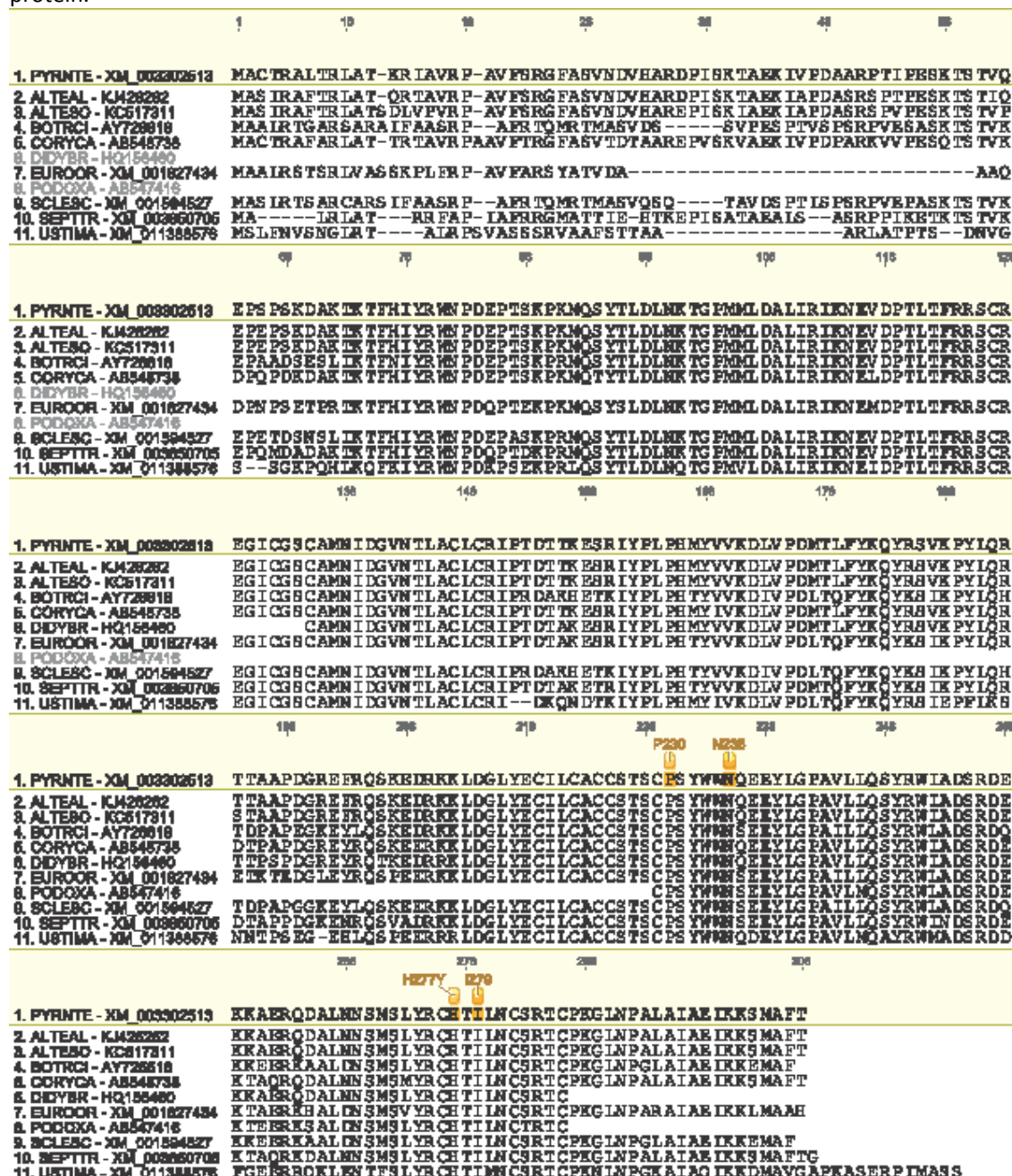


Figure 5. Amino acid sequence alignment of the SdhB family. Sequences are named by species EPP0 code and NCBI gene accession number. Residues highlighted in yellow in the archetype sequence from *Pyrenophora teres* f. sp. *teres* denote amino acid substitutions associated with fungicide

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.

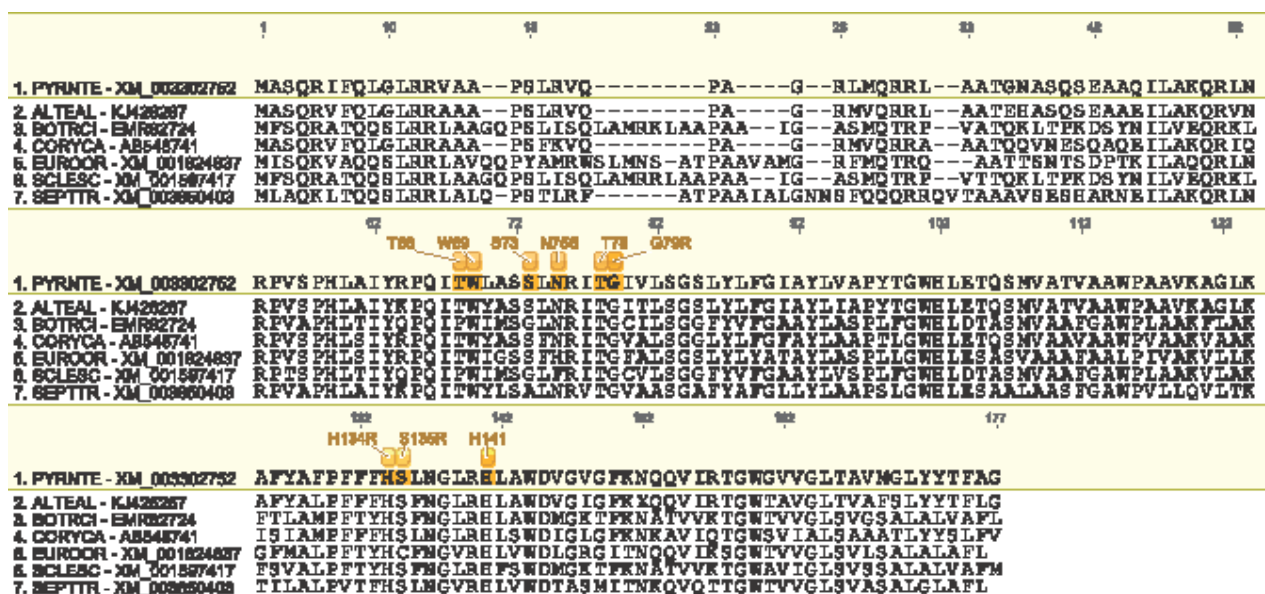


Figure 6. Amino acid sequence alignment of the SdhC family. Sequences are named by species EPPO code and NCBI gene accession number. Residues highlighted in yellow in the archetype sequence from *Pyrenophora teres* f. sp. *teres* denote amino acid substitutions associated with fungicide resistance at 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.

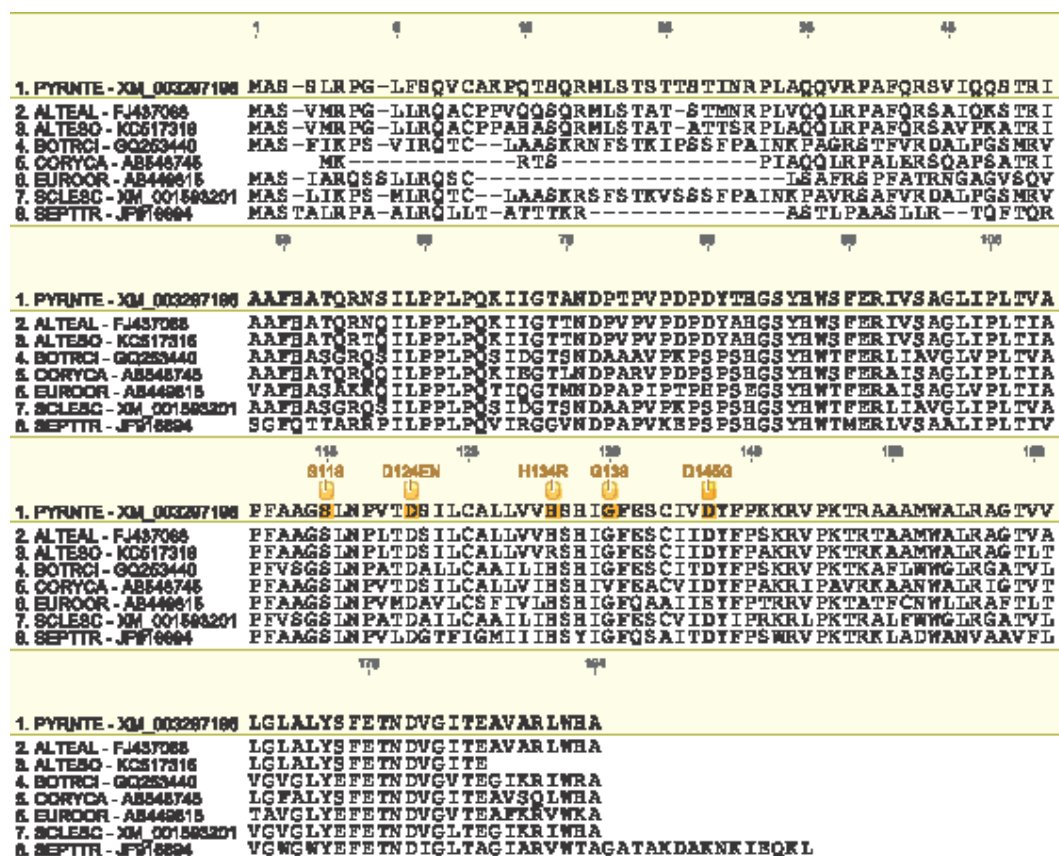


Figure 7. Amino acid sequence alignment of the SdhD family. Sequences are named by species EPPO code and NCBI gene accession number. Residues highlighted in yellow in the archetype sequence from *Pyrenophora teres* f. sp. *teres* denote amino acid substitutions associated with fungicide resistance at 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.

[illegible]

Figure 8. Amino acid sequence alignment of the CesA3 family. Sequences are named by species EPPO code and NCBI gene accession number. Residues highlighted in yellow in the archetype sequence from *Phytophthora infestans* 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

1. BOTRCI - AF435884	MEDSTIAHTTAILQTLALSSIDLPL---LTVNNGN-----KGIRLPGADTAEKALERELAALVSRVQRL	70	74	78	82	86	90	94	98
2. ALTEBI - AY700082	MAAEYSSVSATIRNLRQHDPTRDPSFSAQVSANGAKTAVNATALPGPESDEKTOLOQELSA LCSRIDFL								
3. ALTELO - DQ887638	MAAEYSSVSATIRNLRQHDPTRDPSFSAQVSANGAKTAAATALPGPESSEKTOLEQELSA LCSRIDFL								
4. PLEOAL - EU711371	MAAEYSSVSATIRNLRQHDPTRDPSFSAQVSANGAKTAAATALPGPESSEKTOLEQELSA LCSRIDFL								
1. BOTRCI - AF435884	BARAITVNNQ-----TLPTDTPNELGAPSAFADVLTG---APSRASK-STTSRQQLVNSLLAAREAPTGGG	130	140	150	160	170	180	190	
2. ALTEBI - AY700082	EHKSNHAANQGGQGFPLTPAQEPSPDEGALYTPAGVFRGNNGPPAQGRGSGNKERAIWVSNWLAAKESNGDPE								
3. ALTELO - DQ887638	EHKSNHAANQGGQGFPLTPAQEPSPDEGALYTPAGVFRGNNGPPAQGRGSGNKERAIWVSNWLAAKESNGDPE								
4. PLEOAL - EU711371	EHKSNHAANQGGQGFPLTPAQEPSPDEGALYTPAGVFRGNNGPPAQGRGSGNKERAIWVSNWLAAKESNGDPE								
1. BOTRCI - AF435884	RPPKFTKLSDEELEALREHVDHQSKQLDSQKSELAGVHAQLFEQKQRQEQALNVLEVERVAALERELKKHQ	250	260	270	280	290	300	310	320
2. ALTEBI - AY700082	QP--AAALTEEQNLNLRVHLNQADQIRNQRREHIDNLSQEVNKKQLTTQSMVF--EHGIEDIGALKRELKHKHQ								
3. ALTELO - DQ887638	QP--AAALTEEQNLNLRVHLNQADQIRNQRREHIDNLSQEVNKKQLTTQSMVF--EHGIEDIGALKRELKHKHQ								
4. PLEOAL - EU711371	EP--AVALTEEQFNLRVHLNQADQIRNQRREHIDNLSQEVNKKQLTTQSMVF--EHGIEDIGALKRELKHKHQ								
1. BOTRCI - AF435884	QANEAFQKALREIGEIVTAVARGDLSKKVQIHSVEMDPEITTFKRVINTMMDQLQIFSSSEVSRVAREVGTG	370	380	390	400	410	420	430	440
2. ALTEBI - AY700082	QANLAFQKALREIGAIIVTAVAMGDLSKKVLIHAKEMDPPEITLFKRTINTMVDQLQEFASQVTFLLAREVGTG								
3. ALTELO - DQ887638	QANLAFQKALREIGAIIVTAVAMGDLSKKVLIHAKEMDPPEITLFKRTINTMVDQLQEFASQVTFLLAREVGTG								
4. PLEOAL - EU711371	QANLAFQKALREIGAIIVTAVAMGDLSKKVLIHAKEMDPPEITLFKRTINTMVDQLQEFASQVTFLLAREVGTG								
1. BOTRCI - AF435884	GILGGQAKISGVDGTWKKELTDNVNVMAQNLTQDVREIASVTTAVAHGDLTQKIERPAQGEILQLQQTINTM	450	460	470	480	490	500	510	520
2. ALTEBI - AY700082	GRLGGOANLPGVAGIWAELTD--INGMAKNLTQDVREIAVVTAVAMGDLSRKIERPARGEILQLQQTINSM								
3. ALTELO - DQ887638	GRLGGOANLPGVAGIWAELTDSDVNGMAKNLTQDVREIAVVTAVAMGDLSRKIERPARGEILQLQQTINSM								
4. PLEOAL - EU711371	GRLGGOANLPGVAGIWAELTD--INMMANLTQDVREIAVVTAVAHGDLTSRKIERPARGEILQLQQTINTM								
1. BOTRCI - AF435884	VDQLRTFAASVTRVARDVGTSGILGGQAEISGVQGMWNTLIYVNVAMANNLTQVVDIAIVTTAVAKGDLT	540	550	560	570	580	590	600	610
2. ALTEBI - AY700082	VDQLQSFATQVTKVARDVGTSGKLGGOAEIAGVKGGMWNETLVNVNAMAQNLTQVVDIAQVTTAVAQGNLT								
3. ALTELO - DQ887638	VDQLQSFATQVTKVARDVGTSGKLGGOAEIAGVKGGMWNETLVNVNAMAQNLTQVVDIAQVTTAVAQGNLT								
4. PLEOAL - EU711371	VDQLQSFATQVTKVARDVGTSGKLGGOAEIAGVKGGMWNETLVNVNAMAQNLTQVVDIAQVTTAVAQGNLT								
1. BOTRCI - AF435884	QKVQAECKGEIKQLKETINSMVDQLQQFAREVTKIAREVGTSGRLGGQATVHGVGEGTWKDLTENVNGMAMN	630	640	650	660	670	680	690	700
2. ALTEBI - AY700082	RKVEAECKGEILELKNLTINRMVDQLQQFAHEVTKIAREVGTSGRLGGQATVHGVGEGTWKDLTENVNGMAMN								
3. ALTELO - DQ887638	RKVEAECKGEILELKNLTINRMVDQLQQFAHEVTKIAREVGTSGRLGGQATVHGVGEGTWKDLTENVNGMAMN								
4. PLEOAL - EU711371	RKVEAECKGEILELKNLTINRMVDQLQQFAHEVTKIAREVGTSGRLGGQATVHGVGEGTWKDLTENVNGMAMN								
1. BOTRCI - AF435884	LTTQVREIAKVTTAVARGDLTKKIEVEVOGEIASLKDTINTMVDRLSTFAFEVSKVAREVGTGDLGGQAAQ	720	730	740	750	760	770	780	790
2. ALTEBI - AY700082	LTTQVREIAKVTTAVARGDLTKKIEVEVOGEIASLKDTINTMVDRLSTFAFEVSKVAREVGTGDLGGQAAQ								
3. ALTELO - DQ887638	LTTQVREIAKVTTAVARGDLTKKIEVEVOGEIASLKDTINTMVDRLSTFAFEVSKVAREVGTGDLGGQAAQ								
4. PLEOAL - EU711371	LTTQVREIAKVTTAVARGDLTKKIEVEVOGEIASLKDTINTMVDRLSTFAFEVSKVAREVGTGDLGGQAAQ								
1. BOTRCI - AF435884	VDNVEGKWKDLTENVNVTMAQNLTQVRSISEVTTQAIAGKDMSSRRVHVDAEGEIRLLKDTVNDVMRLDEWS	810	820	830	840	850	860	870	880
2. ALTEBI - AY700082	VDNVEGKWKDLTENVNVTMAQNLTQVRSISEVTTQAIAGKDMSSRRVHVDAEGEIRLLKDTVNDVMRLDEWS								
3. ALTELO - DQ887638	VDNVEGKWKDLTENVNVTMAQNLTQVRSISEVTTQAIAGKDMSSRRVHVDAEGEIRLLKDTVNDVMRLDEWS								
4. PLEOAL - EU711371	VDNVEGKWKDLTENVNVTMAQNLTQVRSISEVTTQAIAGKDMSSRRVHVDAEGEIRLLKDTVNDVMRLDEWS								
1. BOTRCI - AF435884	NEVQVAKDVGVGDKMGSGQADVAGIGRWKEITTDVNTMANNTLTTQVRAFCDITNAATDGDFTKLTIVEAS	960	970	980	990	1000	1010	1020	1030
2. ALTEBI - AY700082	LAVKRVARDVGVGDKMGSGQADVARDIDGRWKEITTDVNTMAQNLTQVRAFCDITNAAMEGKFT-QITVEAS								
3. ALTELO - DQ887638	LAVKRVARDVGVGDKMGSGQADVARDIDGRWKEITTDVNTMAQNLTQVRAFCDITNAAMEGKFT-QITVEAS								
4. PLEOAL - EU711371	LAVKRVARDVGVGDKMGSGQADVARDIDGRWKEITTDVNTMAQNLTQVRAFCDITNAAMEGKFT-QITVEAS								
1. BOTRCI - AF435884	GEMDELKRRKINQMVSLLRESIQRNLAAREAAELANKTKSEFLANMSHEIRTPMNGIIGMTQLTDLTDLTQY	1040	1050	1060	1070	1080	1090	1100	1110
2. ALTEBI - AY700082	GEMDELKRRKINQMVSLLRESIQRNLAAREAAELANKTKSEFLANMSHEIRTPMNGIIGMTQLTDLTDLTQY								
3. ALTELO - DQ887638	GEMDELKRRKINQMVSLLRESIQRNLAAREAAELANKTKSEFLANMSHEIRTPMNGIIGMTQLTDLTDLTQY								
4. PLEOAL - EU711371	GEMDELKRRKINQMVSLLRESIQRNLAAREAAELANKTKSEFLANMSHEIRTPMNGIIGMTQLTDLTDLTQY								
1. BOTRCI - AF435884	QREMLNIVHNLANSLLTIIDDLISKIEANRMVMEIEIPYTLRGTVFNALKTLAVKANEFKFLDLYRVDSS	1120	1130	1140	1150	1160	1170	1180	1190
2. ALTEBI - AY700082	QREMLTIVHNLANSLLTIIDDLISKIEANRMVMEIEIPYTLRGTVFNALKTLAVKANEFKFLDLYRVDSS								
3. ALTELO - DQ887638	QREMLTIVHNLANSLLTIIDDLISKIEANRMVMEIEIPYTLRGTVFNALKTLAVKANEFKFLDLYRVDSS								
4. PLEOAL - EU711371	QREMLTIVHNLANSLLTIIDDLISKIEANRMVMEIEIPYTLRGTVFNALKTLAVKANEFKFLDLYRVDSS								
1. BOTRCI - AF435884	VPDHYVVGDSFRLRQIILNLVGNAIKFTHEGVEVSLTIQKAEQDHCAPNEYAVEFCVSDTGIGIQADKLNLI	1200	1210	1220	1230	1240	1250	1260	1270
2. ALTEBI - AY700082	VPDHYVVGDSFRLRQIILNLVGNAIKFTHEGVEVSLTIQKAEQDHCAPNEYAVEFCVSDTGIGIRGDKLNLI								
3. ALTELO - DQ887638	VPDHYVVGDSFRLRQIILNLVGNAIKFTHEGVEVSLTIQKAEQDHCAPNEYAVEFCVSDTGIGIRGDKLNLI								
4. PLEOAL - EU711371	VPDHYVVGDSFRLRQIILNLVGNAIKFTHEGVEVSLTIQKAEQDHCAPNEYAVEFCVSDTGIGIRGDKLNLI								
1. BOTRCI - AF435884	DTFQQAAGSTTRKFGGTLGLSISKRVLTMGGRMWVESDAGKGSVFYFTYKVRGKPAIQAIQPLVAYK	1280	1290	1300	1310	1320	1330	1340	1350
2. ALTEBI - AY700082	DTFQQAAGSTTRKFGGTLGLSISKRVLTMGGRMWVESDAGKGSVFYFTYKVRGKPAIQAIQPLVAYK								
3. ALTELO - DQ887638	DTFQQAAGSTTRKFGGTLGLSISKRVLTMGGRMWVESDAGKGSVFYFTYKVRGKPAIQAIQPLVAYK								
4. PLEOAL - EU711371	DTFQQAAGSTTRKFGGTLGLSISKRVLTMGGRMWVESDAGKGSVFYFTYKVRGKPAIQAIQPLVAYK								

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.

Table 1 CytB

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 Cyp51A

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

Table 3 Cyp51B

Position number based on alignment to reference sequence from *Zymoseptoria tritici* (NCBI gene accession number AY253234).

Amino acid substitution(s) in archetype	Homologous position in other species
T66	A61V in CANDAL
C80	S79T in ERYSGT
D107V	NA
L126	F120L in PHAKPA
D134G	NA
V136A/C/G	NA
Y137F	Y132F/H in CANDAL
	Y132F/H in PHAKPA
	Y134F in PUCCRT
	Y136F in ERYSGH
	Y136F in ERYSGT
	Y136F in MONIFC
	Y136F in MYCOFI
	Y136F in UNCINE
	Y140F/H in SACCCE
	Y145F in FILBNF
M145L	NA
K148	K142R in PHAKPA
	K143E in CANDAL
	K147Q in ERYSGH
V151	I145F in PHAKPA
D176	K175N in ERYSGT
N178S	NA
S208T	NA
N284H	NA
E300	E297K in CERCBE
H303Y	NA
A311G	A313G in MYCOFI
G312A	NA
I333	I330T in CERCBE
A379G	A381G in MYCOFI
I381V	NA
P391	P394S in CERCBE
A410T	S405F in CANDAL
G412A	NA
H430	H399P in ASPEFL
A453	D411N in ASPEFL
Y459C/D/N/S/P/Δ	Y461D in MYCOFI
G460D/Δ	G462A in MYCOFI
Y461D/H/S	F449S in CANDAL
	Y463D/H/N in MYCOFI
G476	G464S in CANDAL
	G484S in FILBNF

R479	R467K in CANDAL
I483	I471T in CANDAL
	I475T in PHAKPA
V490L	NA
T496	T454P in ASPEFL
G510C	NA
S524T	S508T in PYRPBR
	S509T in ERYSGH

Table 4 *b*-Tubulin

Position number based on alignment to reference sequence from *Aspergillus nidulans* (benA) (NCBI gene accession number M17519).

Amino acid substitution(s) in archetype	Homologous position in other species
H6L/Y	H6Y in LEPTNO
	H6Y in MONIFC
Y50N/S	Y50N in GIBBFU β_1 -tubulin
	Y50C in GIBBZE β_2 -tubulin
	Y50C in HYPMOD
M73	Q73R in GIBBZE β_2 -tubulin
Q134K	NA
A165V	NA
F167	F167Y in CERCBE
	F167Y in COCHHE
	F167Y in GIBBZE β_2 -tubulin
	F167Y in NEUSCR
	F167Y in PENIEX
E198D/K/Q	E198A/G/K/V in BOTRCI
	E198A in CERCBE
	E198V in GIBBFU β_2 -tubulin
	E198K/L/Q in GIBBZE β_2 -tubulin
	E198A/Q 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 β_2 -tubulin
	F200Y in GIBBZE β_2 -tubulin
	F200Y in PENIAU
	F200Y in PENIIT
	F200Y in RHYNSE
	F200Y in VENTIN
L240	L240F in MONILA
	L240F in PYRPBR
	L240F in VENTIN
M257L	NA

Table 5 SdhB

Position number based on alignment to reference sequence from *Pyrenophora teres* f. sp. *teres* (NCBI gene accession number XM_003302513).

Amino acid substitution(s) in archetype	Homologous position in other species
P230	P225F/L/T in BOTRCI
N235	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->Y in PODOXA ^a
I279	I269V in SEPTTR

^aAmino acid position number for this substitution is unknown as only 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

Table 8 CesA3

Position number based on alignment to reference sequence from *Phytophthora infestans* (NCBI gene accession number EF563995).

Amino acid substitution(s) in archetype	Homologous position in other species
Q1077	Q1077K in PHYTCP
G1105A/V	G1105S/V in PLASVI
	G1105V/W in PSPECU
V1109L	V1109L/M in PHYTCP
	V1109L in PHYTDR

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
I273	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

Table 10 Abbreviations of Species Names

Abbreviation (EPPO code)	Name of pathogen
AJELCP	<i>Ajellomyces capsulatus</i>
ALTEAL	<i>Alternaria alternata</i>
ALTEBI	<i>Alternaria brassicicola</i>
ALTELO	<i>Alternaria longipes</i>
ALTELY	<i>Alternaria arborescens</i>
ALTESO	<i>Alternaria solani</i>
ALTETO	<i>Alternaria tomato</i>
ASPEFL	<i>Aspergillus flavus</i>
ASPEFU	<i>Aspergillus fumigatus</i>
ASPEND	<i>Emericella nidulans</i>
ASPEPA	<i>Aspergillus parasiticus</i>
BOTRCI	<i>Botryotinia fuckeliana</i>
CANDAL	<i>Candida albicans</i>
COCHHE	<i>Cochliobolus heterostrophus</i>
COLLGR	<i>Glomerella graminicola</i>
CORYCA	<i>Corynespora cassiicola</i>
CERCBE	<i>Cercospora beticola</i>
DIDYBR	<i>Stagonosporopsis cucurbitacearum</i>
ERYSGH	<i>Blumeria graminis</i> f. sp. <i>hordei</i>
ERYSGT	<i>Blumeria graminis</i> f. sp. <i>tritici</i>
EUROOR	<i>Eurotium oryzae</i>
FILBNF	<i>Filobasidiella neoformans</i>
GIBBFU	<i>Gibberella fujikuroi</i>
GIBBZE	<i>Gibberella zeae</i>
HELMSO	<i>Helminthosporium solani</i>
HYPMOD	<i>Hypomyces odoratus</i>
LEPTNO	<i>Parastagonospora nodorum</i>
MONGNI	<i>Monographella nivalis</i>
MONIFC	<i>Monilinia fructicola</i>
MONILA	<i>Monilinia laxa</i>
MICDMA	<i>Microdochium majus</i>
MYCOFI	<i>Mycosphaerella fijiensis</i>
MYCORA	<i>Didymella rabiei</i>
NEUSCR	<i>Neurospora crassa</i>
PENIAU	<i>Penicillium aurantiogriseum</i>
PENIEX	<i>Penicillium expansum</i>
PENIIT	<i>Penicillium italicum</i>
PHAKPA	<i>Phakopsora pachyrhizi</i>
PHYTCP	<i>Phytophthora capsici</i>
PHYTDR	<i>Phytophthora drechsleri</i>
PHYTIN	<i>Phytophthora infestans</i>
PLASVI	<i>Plasmopara viticola</i>
PLEOAL	<i>Pleospora allii</i>
PODOFU	<i>Podosphaera fusca</i>
PODOXA	<i>Podosphaera xanthii</i>
PSPECU	<i>Pseudoperonospora cubensis</i>
PUCCRT	<i>Puccinia trititica</i>
PYRIOR	<i>Magnaporthe oryzae</i>

PYRNTE	<i>Pyrenophora teres</i>
PYRNTR	<i>Pyrenophora tritici-repentis</i>
PYRPBR	<i>Pyrenopeziza brassicae</i>
RHIZSO	<i>Thanatephorus cucumeris</i>
RHYNSE	<i>Rhynchosporium secalis</i>
SACCCE	<i>Saccharomyces cerevisiae</i>
SCLEHO	<i>Sclerotinia homoeocarpa</i>
SCLESC	<i>Sclerotinia sclerotiorum</i>
SEPTTR	<i>Zymoseptoria tritici</i>
UNCINE	<i>Erysiphe necator</i>
USTIMA	<i>Ustilago maydis</i>
VENTIN	<i>Venturia inaequalis</i>