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

Wesley Mair, Francisco Lopez-Ruiz, Gerd Stammler, William Clark, Fiona Burnett, Derek Hollomon, Hideo Ishii, Tarlochan S Thind, James KM Brown, Bart Fraaije, Hans Cools, Michael Shaw, Sabine Fillinger, Anne-Sophie Walker, Emilia Mellado, Guido Schnabel, Andreas Mehlo and Richard P Oliver a*

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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1 BACKGROUND

Evolved resistance to fungicides is a major problem limiting our ability to control agricultural, medical and veterinary pathogens. 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 or to overexpression of target genes, 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, 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. 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 and their target proteins. These are C3 and cytochrome b (Cytb) (Table 1); G1 and two sterol C14-demethylases (paralogues Cyp51A and Cyp51B) (Table 2

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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 PYROR                       |
|                                       | F129L in PYRNT                       |
|                                       | F129L in RHEZSO                      |
| G137                                   | G137 in PHAKPA                       |
| G143A                                  | G143A in ALTEAL                      |
|                                       | G143A in ALTELY                      |
|                                       | G143A in ALTEGO                      |
|                                       | G143A in ALTEO                       |
|                                       | G143A in CERCLE                      |
|                                       | G143A in CERCBE                      |
|                                       | G143A in COLGGR                      |
|                                       | G143A in ERSIGTO                     |
|                                       | G143A in LEPTANO                     |
|                                       | G143A in MICROMA                     |
|                                       | G143A in MONGNI                      |
|                                       | G143A in MYCOFI                      |
|                                       | G143A in MYCORA                      |
|                                       | G143A in PLASVI                      |
|                                       | G143A in DCOSFI                      |
|                                       | G143A in PODOFU                      |
|                                       | G143A in PSPECU                      |
|                                       | G143A in PYROR                       |
|                                       | G143A in PYRNT                       |
|                                       | G143A in RHEZSO                      |
|                                       | 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 AELCP                      |
| 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                                  |
| P393L                                  | NA                                  |
| Y431C                                  | NA                                  |
| G432S                                  | NA                                  |
| G434C                                  | NA                                  |
| T440A                                  | NA                                  |
| G448S                                  | NA                                  |
| T470                                   | T469S in ASPEFL                      |
| Y491H                                  | NA                                  |
| F495I                                  | NA                                  |

and 3); B1/B2 and β-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) (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
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Table 3. Cyp51B. Position number based on alignment to reference sequence from Zymoseptoria tritici (NCBI gene accession number AY255234) (Amino acid substitution(s) in archetype | Homologous position in other species)

| 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                    |
|                                       | Y131F/H in ERYSGT                   |
|                                       | Y134F in PUCCRT                     |
|                                       | Y136F in ERYSGT                     |
|                                       | Y136F in MYCOFI                     |
|                                       | Y136F in UNCINE                     |
|                                       | Y140F/H in SACCECE                  |
|                                       | YA155F in FILBNF                    |
| M145L                                 | NA                                  |
| K148                                  | K142R in PHAKPA                     |
| V151                                  | K143E in CANDAL                      |
| D176                                  | K147Q in ERYSGT                     |
| N178S                                 | IA155F in PHAKPA                    |
| 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                                  | P3845 in CERCBE                     |
| A410T                                 | S405F in CANDAL                     |
| G412A                                 | NA                                  |
| H430                                  | H399P in ASPEFL                      |
| A453                                  | D411N in ASPEFL                     |
| Y459C/D/N/S/P/Δ                       | Y4610D in MYCOFI                    |
| G460D/Δ                               | G462A in MYCOFI                     |
| Y461D/H/S                             | F449S in CANDAL                     |
|                                       | Y463D/H/N in MYCOFI                 |
| G476                                  | G4645 in CANDAL                     |
|                                       | G4845 in FILBNF                     |
| R479                                  | R467K in CANDAL                     |
| H43                                     | I471T in CANDAL                     |
| Y490L                                 | I475T in PHAKPA                      |
| T496                                  | NA                                  |
| G510C                                 | T454P in ASPEFL                     |
| S524T                                 | NA                                  |
|                                        |                                       |

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 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 to 3).

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)

| Amino acid substitution(s) in archetype | Homologous position in other species |
|----------------------------------------|-------------------------------------|
| H6LY                                  | H6Y in LEPTNO                       |
|                                       | H6Y in MONIFC                       |
| Y50N/S                                | Y50N in GIBBFE β₁-tubulin            |
|                                       | Y50C in GIBBZE β₂-tubulin            |
|                                       | Y50C in HYPMOD                      |
| M73                                   | Q73R in GIBBZE β₂-tubulin            |
| Q134K                                 | NA                                  |
| A165V                                 | NA                                  |
| F167                                  | F167Y in CERCBE                     |
|                                       | F167Y in COCHHE                     |
|                                       | F167Y in MONIFC                     |
|                                       | F167Y in PENC1                       |
|                                       | E198A/G/V in BOTRCI                 |
|                                       | E198A in CERCBE                     |
|                                       | E198V in GIBBFE β₂-tubulin           |
|                                       | E198K/L/Q in GIBBZE β₂-tubulin       |
|                                       | E198A/V in HELMSO                    |
|                                       | E198A/K/MONIFC                      |
|                                       | E198G in PENCE                      |
|                                       | E198A/K/PENAU                       |
|                                       | E198K/V/PENEX                       |
|                                       | E198K in SCLEHO                     |
|                                       | E198A/K in VENTIN                    |
| F200Y                                 | F200Y in BOTRCI                     |
|                                       | F200Y in GIBBFE β₂-tubulin           |
|                                       | F200Y in GIBBZE β₂-tubulin           |
|                                       | F200Y in PENIAU                      |
|                                       | F200Y in PENIT                       |
|                                       | F200Y in RHYNSE                     |
|                                       | F200Y in VENTIN                     |
| L240                                  | L240F in MONILA                     |
|                                       | L240F in PYPBR                      |
|                                       | 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 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 BORCI H277R/Y  |                                      |
| in ALTEAL H277R/Y in ALTESO H277R/Y  |                                      |
| in DIDYBR H278R/Y in CORYCA H → Y in  |                                      |
| PODOXAN° I269V in SEPTTR               |                                      |

° 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                    |                                      |
| N75S N86K/S in SEPTTR                 |                                      |
| T78 T90I in EUROOR                    |                                      |
| G79R G90R in SEPTTR                   |                                      |
| H134R H134R in ALTEAL                 |                                      |
| H135R H135R in ALTESO                 |                                      |
| H133R H133R in SCLESC                 |                                      |
| S118 S89P in CORYCA                   |                                      |
| S118 S89P in CORYCA                   |                                      |
| D124E/N NA                            |                                      |
| H134R H134R in ALTEAL                 |                                      |
| G138 D145G                            |                                      |
| D129E 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 H134R in BOTRCI                 |                                      |
| H133R H133R in ALTEAL                 |                                      |
| G138 D145G                            |                                      |
| D129E in SEPTTR                       |                                      |

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.
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**Figure 2.** Amino acid sequence alignment of the Cyp51A family. Sequences are named by 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 *Phytophthora infestans* (NCBI gene accession number EF563995)

| Amino acid substitution(s) in archetype | Homologous position in other species |
|----------------------------------------|-------------------------------------|
| Q1077                                  | Q1077K in PHYTCP                     |
| G1105A/V                               | G1055S/V in PLASVI                   |
| V1109L                                 | G1105V/W in PSEPUC                   |
|                                        | V1109L/M in PHYTCP                   |

**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/P                              | NA                                  |
| V368F                                  | NA                                  |
| Q369H/P                                | NA                                  |
| N373S                                  | NA                                  |
| G403                                   | G420D in ALTELO                     |
| T447                                   | NA                                  |
| E738                                   | E753K in ALTEBI                     |
| T750                                   | T765R in PLEOAL                     |
| Q762                                   | Q777R in PLEOAL                     |
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 β-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.
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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 these 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 *ASPFEFU* (*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.

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.10,11 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.
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.
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\(^8,12\)\(^{–}15\) using Geneious 6.1.8 software (Biomatters). Alignments of sequences were generated using the ClustalW\(^{16}\) 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 eflux 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\(_1\) 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 Würsig 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, Henniquin 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).