Molecular Epidemiology of Azole-Resistant *Aspergillus fumigatus* in Sawmills of Eastern France by Microsatellite Genotyping

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**Abstract:** Background: Wood chipping has been described as a potential hotspot for the selection of azole-resistant *Aspergillus fumigatus* (AR Af). We previously reported AR Af isolates in sawmills (Eastern France), most of which contained the TR 34/L98H mutation. **Methods:** To study genotypic relatedness, microsatellite genotyping (short tandem repeat for *A. fumigatus* (STR Af)) was performed on 41 azole-susceptible *A. fumigatus* (AS Af) and 23 AR Af isolated from 18 sawmills and two clinical *A. fumigatus* (sensitive and resistant) isolated from a sinus sample of a woodworker. **Results:** Fifty-four unique multilocus genotypes (MLGs) were described among the 66 isolates: 13/24 AR Af and 41/42 AS Af. Allelic diversity was higher for AS Af than for AR Af. Among the 24 AR Af, five isolates had their own MLGs. Thirteen AR Af (54%) belonged to the same group, composed of four close MLGs, defined using Bruvo’s distance. Thirty-two of the 42 AS Af (76%) had their own MLGs and could not be grouped with the Bruvo’s distance cutoff used (0.2). **Conclusion:** Thus, at a regional scale and in the particular environment of the wood industry, common but also different distinct genotypes, even in the same sawmill, were identified. This suggests that the hypothesis of AR Af clonal expansion from a common strain is probably insufficient to explain genotype emergence and distribution.

**Keywords:** *Aspergillus fumigatus*; azole-resistant; sawmill; microsatellite; multilocus genotype

1. Introduction

*Aspergillus fumigatus* is a saprophytic fungus that is widespread in the environment with an ecological niche of decaying vegetation and soil [1]. It is also a ubiquitously opportunistic pathogen responsible for aspergillosis, notably invasive aspergillosis (IA)—the most severe form of the disease [2]. Indeed, the IA mortality rate is high and can reach 65% [3].

Although azole antifungals improve the management of *Aspergillus*-disease, this clinical advance might be threatened by the emergence of azole-resistant *A. fumigatus* (AR Af) worldwide [4,5]. Over the past 20 years, there have been increasing reports of AR Af recovered on the five continents both in clinical and environmental samples [4]. The environmental route of resistance, based on the wide scale use of azole fungicides in the environment, plays a significant role [6]. Two main mechanisms of resistance that are probably of environmental origin, TR 34/L98H and TR 34/Y121F/T289A mutations, have commonly been described in strains from azole-naïve patients and from the environment [4,5].

Over the past few years, several environmental areas have been reported as potential hotspots for the selection of AR Af. In the Netherlands, composts containing azole residues constitute a potential hotspot for the emergence of new mutations conferring resistance [7]. Recently, wastes
originating from flower bulbs, green materials, and wood chippings have been reported to be three new hotspots containingazole fungicides with the highest proportion of AR\(\text{Af}\), as compared to wheat cereals, animal manure, grain, maize silage, fruit storage or regional and exotic fruit waste [8]. AR\(\text{Af}\) have been identified in wood environments for some years now. In fact, AR\(\text{Af}\) carrying TR\(34/\L98H\) and TR\(46/Y121F/T289A\) have been described in wood debris of tree trunk hollows in Tanzania and Romania [9]. We also previously described the presence of AR\(\text{Af}\) in the wood environment, namely sawmills of Eastern France, most of which carried the TR\(34/L98H\) mutation [10]. In this previous study, the presence of AR\(\text{Af}\) carrying the TR\(34/L98H\) mutation seemed to greatly depend on theazole fungicide formulation and the quantity in substrates [10].

Consequently, theazole fungicide selection pressure could occur in different environments and in different countries and seems to play an important role in the selection of AR\(\text{Af}\). Although typing of \(A.\ fumigatus\) isolates could provide insights into the dynamics ofazole resistance development, the origin and global diffusion ofazole-resistant \(A.\ fumigatus\) with TR\(34/L98H\) and TR\(46/Y121F/T289A\) are still unclear [11,12]. Currently, short tandem repeat for \(A.\ fumigatus\) assay (STR\(\text{Af}\)), based on a nine microsatellite analysis, is accepted as the reference and robust typing method for this species [13,14].

The aim of this study was to genotype AR\(\text{Af}\) and azole-susceptible \(A.\ fumigatus\) (AS\(\text{Af}\)), previously isolated from sawmills of Eastern France, by using STR\(\text{Af}\) typing in order to study their genetic relatedness and to describe the potential clustering of isolates according to their susceptibility profile [10]. In addition, the clinical isolates (sensitive and TR\(34/L98H\)) previously described in an immunocompetent woodworker with invasive sinusal aspergillosis were compared to the sawmill isolates [15].

2. Materials and Methods

2.1. Collection of \(A.\ fumigatus\) Strains

A total of 64 environmental \(A.\ fumigatus\) strains, isolated from 18 French sawmills (Eastern France) between September 2014 and April 2016, were analyzed (Table 1). Among them, 23 AR\(\text{Af}\) isolates had previously been isolated by using two selective homemade malt extract agar (ThermoFisher, Waltham, MA, USA) media containingazole antifungals: itraconazole called “Maltitra” and voriconazole called “Maltvori” [10]. The 41 selected AS\(\text{Af}\) isolates matched according to the location of AR\(\text{Af}\) isolates for each sawmill. Two clinical isolates (one AR\(\text{Af}\) and one AS\(\text{Af}\)), isolated from sinus samples in an immunocompetent woodworker who developed an invasive sinusal aspergillosis following facial injuries from a work-related accident, were also included (Table 1) [15].

All \(A.\ fumigatus\) isolates were identified to the species level by PCR amplification and sequencing a part of the highly conserved \(\beta\)-tubulin gene [10].

For all AR\(\text{Af}\), minimal inhibitory concentrations (MIC) of four medical azoles (itraconazole, voriconazole, posaconazole and isavuconazole) and two azoles used in agriculture and sawmills (propiconazole and tebuconazole) were obtained by using the EUCAST (European Committee on Antimicrobial Susceptibility) microdilution method [16].

Amplification and sequencing of the \(cyp51A\) gene were performed as previously described [10]. Among the 23 environmental AR\(\text{Af}\), 20 carried the TR\(34/L98H\) mutation, two the TR\(34/L98H/S297T/F495I\) mutation and one the P216L mutation [10]. The clinical AR\(\text{Af}\), one of the two isolates recovered in the woodworker, also carried the TR\(34/L98H\) mutation [15].

MIC of voriconazole and itraconazole for environmental and clinical AS\(\text{Af}\) isolates were measured by the EUCAST method [16].
Table 1. Characteristics of analyzed azole-resistant (AR *A. fumigatus*) and azole-susceptible (AS *A. fumigatus*).

| Isolate Identification | Date of Isolation | Origin | Susceptible or Resistant and Cyp51A Mutation |
|------------------------|-------------------|--------|---------------------------------------------|
| A25                    | February 2015     | Substrate, sawmill A | Resistant, TR34/L98H |
| D2                     | January 2016      | Substrate, sawmill D | Resistant, TR34/L98H |
| D3                     | January 2016      | Substrate, sawmill D | Resistant, TR34/L98H |
| D4                     | January 2016      | Substrate, sawmill D | Resistant, TR34/L98H |
| D5                     | January 2016      | Substrate, sawmill D | Resistant, TR34/L98H/S297T/F495I |
| D6                     | January 2016      | Substrate, sawmill D | Resistant, TR34/L98H |
| D7                     | January 2016      | Substrate, sawmill D | Resistant, TR34/L98H |
| D8                     | January 2016      | Substrate, sawmill D | Resistant, TR34/L98H |
| F13                    | February 2016     | Substrate, sawmill F | Resistant, TR34/L98H |
| F14                    | February 2016     | Substrate, sawmill F | Resistant, TR34/L98H/S297T/F495I |
| G15                    | February 2016     | Substrate, sawmill G | Resistant, TR34/L98H |
| H16                    | February 2016     | Substrate, sawmill H | Resistant, TR34/L98H |
| K17                    | March 2016        | Substrate, sawmill K | Resistant, TR34/L98H |
| M18                    | March 2016        | Substrate, sawmill M | Resistant, TR34/L98H |
| O19                    | March 2016        | Substrate, sawmill O | Resistant, TR34/L98H |
| O20                    | March 2016        | Substrate, sawmill O | Resistant, TR34/L98H |
| P11                    | September 2014    | Substrate, sawmill P | Resistant, TR34/L98H |
| P12                    | September 2014    | Substrate, sawmill P | Resistant, TR34/L98H |
| Q10                    | January 2016      | Substrate, sawmill R | Resistant, TR34/L98H |
| R21                    | April 2016        | Substrate, sawmill S | Resistant, TR34/L98H |
| R22                    | April 2016        | Substrate, sawmill S | Resistant, TR34/L98H |
| S23                    | April 2016        | Substrate, sawmill T | Resistant, TR34/L98H |
| S24                    | April 2016        | Substrate, sawmill T | Resistant, TR34/L98H |
| A175                   | October 2013      | Wood-worker | Clinical |
| A176                   | February 2015     | Substrate, sawmill A | Susceptible |
| A177                   | February 2015     | Substrate, sawmill A | Susceptible |
| A178                   | February 2015     | Substrate, sawmill A | Susceptible |
| C1P                    | January 2016      | Substrate, sawmill C | Susceptible |
| D156                   | January 2016      | Substrate, sawmill D | Susceptible |
| D157                   | January 2016      | Substrate, sawmill D | Susceptible |
| D158                   | January 2016      | Substrate, sawmill D | Susceptible |
| D159                   | January 2016      | Substrate, sawmill D | Susceptible |
| D161                   | January 2016      | Substrate, sawmill D | Susceptible |
| D162                   | January 2016      | Substrate, sawmill D | Susceptible |
| D163                   | January 2016      | Substrate, sawmill D | Susceptible |
| D164                   | January 2016      | Substrate, sawmill D | Susceptible |
| E21I                   | January 2016      | Substrate, sawmill E | Susceptible |
| F19P                   | February 2016     | Substrate, sawmill F | Susceptible |
| F20P                   | February 2016     | Substrate, sawmill F | Susceptible |
| F163                   | February 2016     | Substrate, sawmill F | Susceptible |
| G24P                   | February 2016     | Substrate, sawmill G | Susceptible |
| H22P                   | February 2016     | Substrate, sawmill H | Susceptible |
| H23P                   | February 2016     | Substrate, sawmill H | Susceptible |
| H164                   | February 2016     | Substrate, sawmill H | Susceptible |
| I15P                   | February 2016     | Substrate, sawmill I | Susceptible |
| J16P                   | March 2016        | Substrate, sawmill J | Susceptible |
| K17P                   | March 2016        | Substrate, sawmill K | Susceptible |
| K165                   | March 2016        | Substrate, sawmill K | Susceptible |
| L4P                    | March 2016        | Substrate, sawmill L | Susceptible |
| M5P                    | March 2016        | Substrate, sawmill M | Susceptible |
| N6P                    | March 2016        | Substrate, sawmill N | Susceptible |
| O7P                    | March 2016        | Substrate, sawmill O | Susceptible |
| O8P                    | March 2016        | Substrate, sawmill O | Susceptible |
| O166                   | March 2016        | Substrate, sawmill O | Susceptible |
| P173                   | September 2014    | Substrate, sawmill P | Susceptible |
| P174                   | September 2014    | Substrate, sawmill P | Susceptible |
| Q17P                   | January 2016      | Substrate, sawmill R | Susceptible |
| R11P                   | April 2016        | Substrate, sawmill S | Susceptible |
| R12P                   | April 2016        | Substrate, sawmill S | Susceptible |
| R13P                   | April 2016        | Substrate, sawmill S | Susceptible |
| S13P                   | April 2016        | Substrate, sawmill T | Susceptible |
| S14P                   | April 2016        | Substrate, sawmill T | Susceptible |
| S169                   | April 2016        | Substrate, sawmill T | Susceptible |
| S179                   | April 2016        | Substrate, sawmill T | Susceptible |
| Wood-worker            | July 2013         | Clinical | Susceptible |
2.2. Short Tandem Repeat for A. fumigatus (STRAf) Typing and Analysis

STRAf typing is based on the amplification of nine highly polymorphic microsatellite markers (STRAf 2A, 2B, 2C, 3A, 3B, 3C, 4A, 4B, 4C). These nine STRAf loci were amplified with three triplexed PCRs as previously described [13]. PCR products were diluted 10-fold with water for molecular biology and then analyzed on the Applied Biosystems 3130 Genetic Analyzer (ThermoFisher, Waltham, MA, USA) with GeneScan™ 400 HD ROX™ size standard according to the manufacturer’s instructions (Thermofisher®, Waltham, MA, USA).

Amplicon sizes were determined with GeneMapper software (version 5, ThermoFisher, Waltham, MA, USA) and then transformed in repeat number. STRAf analysis was then performed using RStudio software (version 3.2.2, Boston, MA, USA). Microsatellite genotype distances were calculated using Bruvo’s distance (cutoff value = 0.2) and a minimum spanning network was calculated via the bruvo.msn function on the poppr library [17]. Finally, allelic diversity was calculated for the nine STRAf loci for ARAf and ASAf isolates by using the Simpson index of diversity (D).

3. Results

A total of 54 STRAf unique multilocus genotypes (MLGs) were described among the 66 typed A. fumigatus: 13 for the 24 ARAf and 41 for the 42 ASAf. None of the ARAf isolates had MLGs close to those of the ASAf isolates, and the sensitive clinical isolate identified in the woodworker before the resistant one revealed a completely different MLG.

The nine microsatellite markers including STRAf genotypes, allele count, repeat range, median repeat number and allelic diversity are summarized in Table 2. All loci were polymorphic with a number of alleles ranging from 3 (loci 3B and 4B) to 11 (locus 3A) and from 9 (locus 4B) to 19 (loci 3A and 3C) for ARAf and ASAf isolates, respectively. Loci 3A and 3C displayed the highest polymorphism and allelic diversity for both ARAf and ASAf isolates, whereas locus 4B was the least discriminating with the lowest number of alleles (Table 2). For all microsatellite markers, allelic diversity was higher for ASAf isolates (average $D = 0.811$) than for ARAf isolates (average $D = 0.518$) (Table 2).

| Microsatellite Marker | Azole-Resistant A. fumigatus (ARAf, $n = 24$) | Azole-Susceptible A. fumigatus (ASAf, $n = 42$) |
|-----------------------|-----------------------------------------------|-----------------------------------------------|
|                       | N Alleles | Repeat Range | Median Size | Diversity (D) | N Alleles | Repeat Range | Median Size | Diversity (D) |
| 2A                    | 5         | 13–26        | 14          | 0.507         | 2A         | 11          | 10–27       | 18          | 0.768         |
| 2B                    | 4         | 10–24        | 21          | 0.462         | 2B         | 9           | 12–25       | 19          | 0.766         |
| 2C                    | 4         | 8–16         | 8           | 0.552         | 2C         | 11          | 8–20        | 12          | 0.868         |
| 3A                    | 11        | 10–119       | 32          | 0.872         | 3A         | 19          | 10–49       | 26          | 0.909         |
| 3B                    | 3         | 8–11         | 8           | 0.403         | 3B         | 10          | 8–22        | 9           | 0.771         |
| 3C                    | 7         | 6–32         | 6           | 0.649         | 3C         | 19          | 6–45        | 18          | 0.924         |
| 4A                    | 5         | 5–18         | 8           | 0.361         | 4A         | 13          | 7–26        | 9           | 0.774         |
| 4B                    | 3         | 7–10         | 10          | 0.392         | 4B         | 8           | 5–26        | 9           | 0.746         |
| 4C                    | 5         | 5–30         | 20          | 0.465         | 4C         | 9           | 5–36        | 7           | 0.770         |
| Total alleles         | 47        | NR           | NR          | NR            | Total alleles | 109        | NR          | NR          | NR            |
| Average D             | NR        | NR           | NR          | 0.518         | Average D   | NR          | NR          | NR          | 0.811         |

N Alleles = Allele counts; D = Simpson index of diversity; NR = non relevant.

Among the 42 ASAf, 41 MLGs were identified, with two isolates from two different sawmills (A and D, 60 km away) sharing the same genotype (A177 and D159, Figure 1). Thirty-two MLGs (76%) were very distinct and could not be grouped with the Bruvo distance cutoff used. However, four groups of two ASAf were found: D161 and R12P, E21P and O166, S179 and C1P, F20P and F19P.
(genotypes connected by lines in Figure 1). These ASAf were isolated from different sawmills, except for isolates F20P and F19P which were recovered in the same sawmill (F) at different locations.

Figure 1. Minimum spanning network of nine microsatellite loci STRAf using Bruvo’s distance. Letters correspond to sawmill and the following numbers ± letters are genotype numbers. Each circle represents one multilocus genotype, the size of which is proportional to frequency. Different colors represent the different isolates (susceptible or resistant, and their mutations on the Cyp51A gene and its promoter). Link thickness is proportional to genotype similarity.

Isolates
- Susceptible
- Resistant: TR34/L98H
- Resistant: TR34/L98H/S297T/F4951
- Resistant: P216L

Isolates/Node
- 5
- 3
- 1

DISTANCE
Distance Bruvo / Network (cutoff < 0.2)
Among the 24 ARAf, 13 MLGs were characterized. Four isolates with the TR$_{34}$/L98H mutation (genotype numbers Q10, M18, D8 and F13, Figure 1) and one with the P216L mutation (genotype S24) had their own MLGs and could not be grouped with Bruvo’s distance. The others were recovered for several isolates grouped in the same circles within the minimum spanning network (Figure 1).

For TR$_{34}$/L98H isolates, two MLGs that were genotypically distinct from others were identified: genotypes D3 and D4 with the TR$_{34}$/L98H mutation, found in the same soil sample, and D5 and F14 with the TR$_{34}$/L98H/S297T/F495I, taken from two geographically distant sawmills (170 km).

Moreover, two groups of MLGs were found with Bruvo’s distance (Figure 1). The first group was composed of four MLGs, including 13/24 ARAf (54%, genotype numbers A25, R21, G15, P12 / D2, D7, S23, O20, P11 / D6, O19, H16 and K17). Among these four closely related STRAf genotypes, three differed by only one repetition of the microsatellite marker 3A; the fourth (K17 ARAf) differed from the three others by the number of repetitions of microsatellite markers 3A and 3C. These four genotypes included TR$_{34}$/L98H ARAf isolated from different sawmills that could be located as far as 200 km from each other; two of these genotypically similar ARAf (P11 and P12) were isolated in one sawmill that imported wood from Russia. The second group consisted of one clinical TR$_{34}$/L98H isolate responsible for invasive sinusal aspergillosis in an immunocompetent woodworker and had a genotype close to a sawmill ARAf also carrying the TR$_{34}$/L98H mutation (genotype R22 in Figure 1). It differed by the number of repetitions on microsatellite markers 3A and 4A. However, the woodworker did not work in sawmill R.

Identical MLGs were thus found in different sawmills, but different MLGs were also found in one sawmill: five different MLGs were identified among the seven ARAf found in sawmill D. This sawmill shared one MLG (genotype D5, mutation TR$_{34}$/L98H/S297T/F495I) with another sawmill. Sawmill D shared MLGs with other sawmills and genotypes that closely resembled each other (genotypes D2, D7 and D6). It also had one MLG (genotypes D3 and D4) that was different from all other MLGs.

4. Discussion

In this study, we reported the presence of both common and different genotypes in ARAf and ASAf from sawmills of Eastern France by using STRAf typing. Our findings are similar to those of several other studies, as we reported that ASAf had a greater genotypic diversity than ARAf [14,18,19]. This could suggest a predominantly clonal expansion of ARAf in the environment. In fact, contrary to ASAf, more than half of ARAf (54%) belonged to the same group with the Bruvo’s distance cutoff used and seemed to be genotypically close. These ARAf came from different sawmills that were geographically far from one another (up to 200 km) and for which a selection pressure by azole fungicide was reported. Direct or indirect contact withazole fungicide was shown, and azole fungicides were detected in substrate samples in these sawmills [10].

This study has some limitations. First, we describe here the genotypic structure of isolates coming from only one region in France and one type of environment. A broader study including genotyping of isolates from other regions, environments and countries would make it possible to know if the genotypes of ARAf and ASAf from Eastern France are shared by isolates in other regions of the world; this could help to improve our understanding of the emergence, diffusion and distribution of ARAf.

In addition, we used STRAf typing which is widely accepted as the reference typing method for A. fumigatus [14]. However, two of the nine microsatellite markers, 3A and 3C, show a lower level of stability which has to be taken into account when interpreting STRAf data during molecular epidemiological analysis [20]. This is illustrated by the greater polymorphisms for these two markers described in our study. Moreover, three genotypes differed by only one repetition on marker 3A and grouped isolates coming from the same sawmill or the same samples (P11 and P12). The difference between these three genotypes might be valid but we cannot exclude a bias in the genotyping method used. Despite the high discriminatory power of STRAf typing, it remains lower than that of whole genome sequencing (WGS). So, it would be interesting to determine whether or not ARAf with the...
same genotype, revealed using STRAf typing, would reveal exactly the same genotype when using WGS. Likewise, exploring genotypic diversity with WGS could be useful.

It is important to emphasize that 13 STRAf genotypes were found among the 24 ARAf. Moreover, some ARAf had their own genotypes and were genotypically distinct from others despite sharing the same mechanism of resistance (mutation TR46/L98H). It is interesting to note that this diversity was reported in one sawmill (D), without any particular characteristics when compared to other sawmills using a dip processing tank, and where five MLGs were reported among the seven ARAf genotyped. These findings contrast with those of some studies describing only one clone of TRAf/L98H ARAf strains [18,21]. The local diversity of the genotype reported here was also highlighted for ARAf isolates in market gardens of the same region in Eastern France. In this study, 22 STRAf genotypes among 44 analyzed ARAf and six groups of genotypically close ARAf were recorded. ARAf isolates could be more diverse in other countries such as Colombia where 19 MLGs were identified in 21 ARAf isolates in the environment [17,22]. Additionally, it has also been shown by STRAf typing that resistant isolates, coming from several countries, belonged to several groups. Similarly, some authors have reported a dispersed structure in ARAf with tandem repeat (TR) mutations [11,14]. Thus, it seems unlikely that the emergence of azole-resistant strains could be the work of a single clone.

The population structure of A. fumigatus is very complex and is likely to be due to several past and current events: historical differentiation, contemporary gene flow, sexual reproduction, recombination, and the localized azole fungicide selection that could drive expansion of ARAf genotypes [11,12]. It was recently reported that certain practices like composting (i.e., stockpiling plant waste) might be the key to resistance selection in A. fumigatus [8]. Some environments supporting the growth, sexual reproduction, genetic variation and containing residues ofazole fungicides could cause these complex mutations to emerge, amplify and spread. So, ARAf may very well emerge in specific environments and spread to other countries due to natural factors such as wind, or anthropogenic factors such as human travel and commercial trade [11,23]. This gene flow could explain the fact that genotypically close ARAf, or those having the same genotype, were found in different countries [18,19,21].

A comparison of genotypes from sawmill ARAf with genotypes from other A. fumigatus isolates in a London database (https://afumid.shinyapps.io/afumID), 4049 A. fumigatus isolates collected worldwide showed that some sawmill ARAf (A25, R21, G15, P12 with the same MLG) presented the same genotype as a clinical Australian isolate (data not shown). Among them, isolate P12 is one of the two isolates found in one sawmill that did not treat wood, but rather imported foreign wood from Russia that had already been treated with fungicides (sawmill P). Curiously, these two ARAf shared the same MLG as other sawmills ARAf. According to these results, the gene flow could not be excluded here and it may be possible that these ARAf emerged in a favorable environment outside of France and then, when imported, spread to the Eastern France sawmills. Conversely, an MLG identified twice in a single sawmill (D) is not linked to any profile in the London database.

The distribution of sawmill ARAf and ASAf genotypes, with a lower diversity of ARAf isolates, also seems to concur with two hypotheses reported by other authors to explain the emergence and distribution of ARAf: the specific genetic background and predisposition of some clones to develop azole-resistance, and the better ability of ARAf isolates to accept the azole-resistant genes via mating and recombination [11,14]. ARAf with TR34/L98H and TR46/Y121F/T289A mutations may have a genetic component that is restricting the resistance genotype to certain strains [24]. It has also been reported that the genetic backgrounds of TR34/L98H and TR46/Y121F/T289A ARAf were less diversified than those of wild-type isolates [24]. This is compatible with selective gene sweeps accompanying the selection of beneficial mutations and the genetic adaptation of A. fumigatus which enable it to survive and reproduce in prevailing or new environments, such as those withazole fungicides [24,25]. Selective sweeps would reduce allelic diversity and one, or a limited number, of clones would predominate locally [23]. The lower allelic and STRAf genotype diversity reported for ARAf isolates from sawmills in our study make this hypothesis likely. So, according to their genetic background, some strains could adapt more easily than others to a given environment and could be more able to develop and persist.
in an environment where the azole fungicide selection pressure is substantial. Moreover, the lower diversity of the ARAf, despite their different origin and the fact that they have been isolated in different sawmills, could suggest that ARAf belonging to the same group (with the Bruvo’s distance cutoff used) are genotypically closed and probably evolved from each other.

In conclusion, in this study we reported both common and different genotypes on a regional scale. Despite having lower allelic diversity than ASAf isolates, some ARAf could not be grouped together with other sawmill ARAf isolates. The diversity of genotypes described here, at a local level, seems to support the multiple origins hypothesis, thus suggesting that the hypothesis of clonal expansion from a common strain is now probably obsolete and insufficient to explain ARAf emergence and distribution. The evolution of ARAf from different origins could also be involved.

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