Equal Distribution of Mating Type Alleles and the Presence of Strobilurin Resistance in Algerian *Zymoseptoria tritici* Field Populations

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Abstract

Zymoseptoria tritici, the causal agent of septoria tritici blotch, is an important wheat pathogen responsible for high yield losses worldwide. The assessment of the distribution of both mating type idiomorphs was studied in several wheat-growing areas in North Algeria. Both mating types occurred at all spatial scales tested and showed an equal frequency distribution at the country level, with 53% for *Mat1-1* and 47% for *Mat1-2*. At finer scales, co-occurrence of both mating types was found in 38% of leaves analyzed and 44% of the studied lesions. Additionally,

efficacy reduction of strobilurin applications prompted us to study strobilurin resistance conferred by the G143A substitution in the mitochondrial cytochrome *b* gene, which was found for the first time in six strains. The results suggest that the Algerian *Z. tritici* population undergoes frequent sexual reproduction mirrored by co-occurrence of both mating types, which likely will rapidly increase the fraction of strobilurin-resistant isolates in the Algerian population, as has been shown in all other regions where strobilurin resistance surfaced.

The foliar wheat pathogen *Zymoseptoria tritici* (Quaedvlieg et al. 2011) causes septoria leaf blotch (STB), which is considered to be one of the most devastating wheat diseases worldwide (Fones and Gurr 2015; Torriani et al. 2015) and occurs every year throughout the major Algerian wheat-growing regions (Benbelkacem et al. 2011). *Z. tritici* is heterothallic with a bipolar mating system (Kema et al. 1996), undergoing both sexual and asexual reproduction. Sexual spores (ascospores) are air-borne and can be dispersed over long distances, whereas asexual spores (pycnidiospores) are dispersed by rain splash over only limited distances (Chen and McDonald 1996; McDonald and Martinez 1990). Several secondary cycles of sexual reproduction occur within a single growing season (Hunter et al. 1999; Kema et al. 1996; Zhan et al. 2003), generating a high degree of genetic variation distributed even at small spatial scales (McDonald and Martinez 1990; Zhan et al. 2002, 2003). The mating type loci of *Z. tritici* have been intensively studied over the last decades (Kema et al. 1996; Siah et al. 2010b; Waalwijk et al. 2002; Zhan et al. 2002), showing their occurrence with equal distribution in most parts of the world as well as in some areas of Algeria (Abrinbana et al. 2010; Alloui et al. 2014; Boukef et al. 2012; Elbekali et al. 2012; Siah et al. 2010b; Zhan et al. 2002). Usually, an equal distribution of the *Mat1-1* and *Mat1-2* idiomorphs in natural field populations is consistent with frequent sexual cycles (Hunter et al. 1999; Zhan et al. 2002). Sexual reproduction generates new genotypes able to circumvent host resistance (Cowger and Mundt 2002) as well as adapt to other selective environments, such as those generated by fungicides. Disease control of STB is mainly based on chemical treatments (Fraaije et al. 2005) such as the chemical group of quinone outside inhibitors (QoI), also known as strobilurins. Due to their broad spectrum (Bartlett et al. 2002) and their additional favorable effects on plant physiology (Ruske et al. 2003), strobilurins were intensively used in STB management. The QoI act by binding to the quinol-oxylizing (Qo) site of the mitochondrial cytochrome *bc1* protein complex, thereby interfering with the mitochondrial respiration in the fungus (Bartlett et al. 2002). However, contrary to expectations, resistance to QoI rapidly surfaced in the United Kingdom in 2001 (Fraaije et al. 2003) and is conferred by a point mutation at codon 143 in the cytochrome

b (*Cytb*) gene, which led to an amino acid exchange from glycine to alanine at position 143 (G143A). This substitution in *Cytb* prevents binding of strobilurins to the Qo site and thereby restores mitochondrial respiration in the fungus (Fraaije et al. 2003; Gisi et al. 2002; Torriani et al. 2009). Intensive deployment of strobilurins over the last decade in Europe boosted the emergence and rapid spread of resistant strains among many countries (Drabešová et al. 2013; Fraaije et al. 2005; Gisi et al. 2005; Siah et al. 2010a; Torriani et al. 2009). In Maghreb countries, sterol demethylation inhibitors (DMI), also known as azoles, are widely used for STB control, and the use of strobilurins is fairly limited. Consequently, *Z. tritici* populations in these countries are supposed to be rather sensitive to strobilurins, supported by the absence of the G143A substitution in both Tunisia and Algeria (Alloui et al. 2013; Boukef et al. 2012; Somai-Jemmali et al. 2014; Stammler et al. 2012). However, recent studies reported the occurrence of QoI resistance in *Z. tritici* at low frequencies in Tunisia (Taher et al. 2014) and Morocco (Elbekali et al. 2011; Siah et al. 2014).

Here, we (i) determined the distribution of both *Z. tritici* mating types in Central and Eastern Algeria over different spatial scales and (ii) identified, for the first time, QoI resistance in these populations.

Materials and Methods

Sampling and strain isolation. A set of 213 *Z. tritici* isolates was obtained in 2013 and 2014 from naturally infected durum wheat fields (*Triticum durum* Desf.) in six different wheat-growing areas of Algeria (Alger, Blida, Tipaza, Constantine, Bouira, and Tipaza) (Fig. 1). Sampling was carried out from three durum wheat cultivars: Vitron, Chen'S, and Ammar 6 (Table 1). Leaves from different plants showing necrotic blotches with pycnidia were collected randomly from one or three fields at each location, and fragments of 5 cm were fixed to glass slides with sticky tape and kept under high-humidity conditions for 24 h. Subsequently, chytrhi from single pycnidia—one or more per lesion from one or more distinct lesions per leaf—were collected with a sterilized needle under a stereomicroscope and transferred to potato dextrose agar (PDA) supplemented with streptomycin (50 mg/liter). After colony appearance, pycnidiospores were spread onto fresh PDA petri plates and kept at room temperature for 4 days; and, finally, single colonies were transferred to fresh PDA plates and stored at 4°C until DNA extraction. In total, 213 isolates were collected, maintained, and analyzed (Table 1).

DNA extraction. Prior to genomic DNA extraction, *Z. tritici* isolates were grown on PDA for 5 days at 18°C. Spore masses were subsequently scraped from the petri dishes and lyophilized in microtubes for 24 h (Epsilon 1-4/2-4 LCS plus; Martin Christ, Osterode am

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Harz, Germany). Two DNA extraction methods were used according to the pathogen population size. For the majority of isolates (192 isolates), DNA was isolated with a KingFisher DNA extraction robot (Thermo Electron Corporation, Breda, The Netherlands). Lyophilized spores were placed in microtubes containing a chrome/steel bead of 3.2 mm (Biospec Products, Bartlesville, OK) and were bead-beaten twice with a Tissue Lyser II (Qiagen, Hilden, Germany) for 20 s at 30 rpm/s. Lysis buffer was added (300 µl) along with 0.5 µl of RNase (2 mg/ml) and the microtube content was once more bead-beaten for 20 s. After 30 min of incubation at 65°C, the microtubes were centrifuged for 10 min at 6,000 rpm (Sigma, Osterode am Harz, Germany). Clear supernatants (200 µl) were collected and used to fill deep well plates containing 520 µl of binding buffer and 60 µl of coated magnetic particles from the beadex Maxi Plant kit (LGCgroup, Berlin). During the process, the particle-bound DNA was washed twice with 400 µl of wash buffer and once with 400 µl of nuclease-free water. Finally, DNA was eluted in 120 µl of elution buffer and quantified with a spectrophotometer (ND-1000, NanoDrop Technologies, Wilmington, DE). For the remaining 20 isolates, the Wizard Magnetic DNA purification System for food Kit (Promega Corp., Madison, WI) was used according to the manufacturer's instructions, with a slight modification. Lyophilized spores (50 mg) were placed in 2-ml tubes with a tungsten carbide bead and shaken in a Mini bead beater (Biospec Products) for 10 s at a maximum speed. Lysis buffer (500 µl) was added and cells were disrupted by another round of bead-beating following the same protocol. The obtained DNA was resuspended in 100 µl of nuclease-free water and quantified as mentioned above.

Polymerase chain reaction mating type and QoI resistance analyses. The mating type of each isolate was identified using a multiplex polymerase chain reaction (PCR) amplification of partial sequences from the two mating type idiomorphs, according to Waalwijk et al. (2002). The specific *Mat1-1* and *Mat1-2* primers (Table 2) generate amplicons of 340 and 660 bp in length for *Mat1-1* and *Mat1-2*, respectively. DNA of the *Z. tritici* *Mat1-1* and *Mat1-2* reference isolates IPO323 and IPO94269, respectively, were included as positive controls.

Sensitivity or resistance for the QoI fungicides among the *Z. tritici* isolates was determined following a mismatch amplification mutation assay, according to Ware (2006). The specific primers (Table 2) generate a 639-bp DNA fragment in sensitive isolates and a 302-bp DNA fragment in resistant isolates. DNA of the standard sensitive *Z. tritici* isolates IPO323 and IPO94269 were used as positive controls. PCRs for mating type analyses were carried out by adding 1 µl of genomic DNA (10 to 20 ng/µl), 5 µl of 10x PCR buffer, 5 µl (600 µM) of dNTP, 0.25 µl (5 U/µl) of Taq DNA Polymerase (Ampli Taq Gold; Roche, Somerville,

MA), and 2.5 µl (6 µM) of each primer to 50 µl of nuclease free water. DNA samples were amplified using an MJ Research PT-100 thermocycler (Bio-Rad, Hercules, CA) programmed at 94°C for 2 min followed by 39 cycles of 94°C for 60 s, 68°C for 30 s, and 72°C for 60 s. The final extension was carried out at 72°C for 10 min.

For QoI analyses, thermal cycling conditions started with initial denaturation at 94°C for 2 min followed by 39 cycles of 60 s at 94°C, 30 s at 55°C when primers pairs for sensitive isolates were used or 30 s at 60°C when primers pairs for resistant isolates were used, before 60 s at 72°C for extension for both sets of PCR. Final PCR cycles were followed by incubation for 10 min at 72°C and cooling-off at 10°C. Amplification products were stained with GelRed, separated in 1% agarose gel, and visualized under UV-light.

Table 1. Origin of *Zymoseptoria tritici* isolates used in this study

Location	N ^a	Cultivar	Year of sampling
Alger	37	Vitron	2013 to 2014
	18	Ammar 6	2014
Blida	9	Vitron	2014
	43	Chen'S	2014
Bouira	13	Waha	2014
Constantine	50	Vitron	2014
Médéa	11	Vitron	2014
Tipaza	32	Vitron	2014
Total	213	...	...

^a Number of isolates.

Table 2. Primers used for *Zymoseptoria tritici* in this study

Primer	Sequence (5' to 3')
MAT1-1 F ^a	CCGCTTTCTGGCTTCTTCGCACTG
MAT1-1 R ^a	TGGACACCATGGTGAGAGAACCT
MAT1-2 F ^a	GGCGCCTCCGAAGCAACT
MAT1-2 R ^a	GATGCGGTTCTGGACTGGAG
StrobSNP2fwd ^b	CTTATGGTCAAATGTCTTTATGATG
StrobSNP1rvs ^b	GGTGACTCAACGTGATAGC
StrobSNPrcF7 ^b	CAATAAGTTAGTTATAACTGTTGCGG
StrobSNPrcR1 ^b	CTATGCATTATAACCCTAGCGT

^a Waalwijk et al. (2002).

^b Ware (2006).

Fig. 1. Map displaying sampling locations of the *Zymoseptoria tritici* populations.

Fungicide sensitivity bioassay. In order to validate the PCR analyses for QoI resistance, colony development of six isolates carrying the QoI-resistant allele and three isolates carrying the QoI-sensitive allele was compared on strobilurin-amended (azoxystrobin at 1 ppm; Amistar; Syngenta, Basel, Switzerland) PDA plates as described earlier (Ware 2006). Isolates (5 µl with a concentration of 4×10^5 spore/ml) were spotted on amended agar plates as well as regular PDA plates amended with 1% methanol as controls. All plates were replicated three times and incubated in the dark at 20°C for 10 days.

Data analyses. The mating type distributions were determined at different spatial scales: within lesions, within leaves, within fields, and within locations. To detect any significant deviation from the expected 1:1 ratio, frequencies of both mating types were analyzed at field and location level using the χ^2 test at the significance level $P = 0.05$, and the distributions between locations were tested using Pearson's χ^2 test.

Results

Mating types determination. In total, 213 *Z. tritici* isolates were obtained (Table 1) and subsequently assayed for *Mat1-1* and *Mat1-2* frequencies. The mating-type-specific PCR generated a single amplicon of either 340 or 660 bp, corresponding with the *Mat1-1* or *Mat1-2* alleles, respectively, in 208 isolates (Fig. 2).

Macro- and microgeographical distribution of *Z. tritici* *Mat* alleles. Both mating types were found among all *Z. tritici* isolates that were isolated from the six sampled locations. Overall, the 208 tested isolates showed a 1:1 ratio as expected in random mating populations with 110 isolates (53%) *Mat1-1* and 98 *Mat1-2* isolates (47%).

The χ^2 test analysis revealed no significant differences in the frequencies of the two mating types within and among locations (Table 3). Both mating types occurred at equal frequencies in each location, with an absolute equal distribution in Blida (Table 3). One to three fields per location were analyzed and tested for the mating type frequencies with a χ^2 test and the results showed that field 2

Fig. 2. Multiplex polymerase chain reaction for *Mat1-1* or *Mat1-2* mating type determinations in Algerian *Zymoseptoria tritici* isolates. Lanes 1 and 2: specific amplicons generated in the reference isolates IPO94269 (660 bp, *Mat1-2*) and IPO323 (340 bp, *Mat1-1*); lanes 3 to 10: various explanatory field isolates from Alger, Blida, and Constantine; and lane M, 100-bp DNA ladder.

Table 3. Frequency distributions of *Zymoseptoria tritici* mating types within fields and among locations

Location	Field	N ^a	Frequency		χ^2 Values (df)	
			<i>Mat1-1</i>	<i>Mat1-2</i>	Within fields	Among locations
Alger	1	31	0.48	0.52	0.03 (1)	0.51 (5)
	2	13	1.00	0.00	10.28* (1)	...
	3	10	0.20	0.80	3.6 (1)	...
Blida	1	15	0.53	0.47	0.06 (1)	...
	2	15	0.40	0.60	0.6 (1)	...
	3	22	0.55	0.45	0.18 (1)	...
Constantine	1	10	1.00	0.00	10.00* (1)	...
	2	10	0.70	0.30	1.6 (1)	...
	3	28	0.29	0.71	5.14* (1)	...
Tipaza	1	17	0.47	0.53	0.05 (1)	...
	2	14	0.57	0.43	0.28 (1)	...
Bouira	1	12	0.58	0.42	0.33 (1)	...
Médéa	1	11	0.55	0.45	0.39 (1)	...
Total	13	208	0.53	0.47	0.69 (1)	...

^a Number of isolates.

from Alger and fields 1 and 3 from Constantine showed a significant deviation from a 1:1 ratio (χ^2 test, $P = 0.05$). The former fields showed exclusively *Mat1-1* strains.

Occurrence of the two mating types on the same leaf or within the same lesion was observed in each location tested, even in fields with a small sample sizes such as those from Bouira and Médéa (Tables 4 and 5). A fine spatial scale investigation on wheat leaves revealed the coexistence of both mating types in the same lesion in 44% (8 of 18) of the cases whereas, when multiple lesions on the same leaf were analyzed, both mating types were found in 38% (10 of 26) of the leaves. Co-occurrence of both mating types with a 1:1 ratio was observed at either lesion and at each leaf scale.

Table 4. Distribution of *Zymoseptoria tritici* mating types among isolates from the same leaf

Leaf number	Location	No. isolates	<i>Mat1-1</i>	<i>Mat1-2</i>
1	Alger	6	2	4
2	Alger	7	3	4
3	Alger	7	5	2
4	Alger	8	2	4
5	Alger	7	7	0
6	Alger	6	6	0
7	Alger	10	2	8
8	Blida	11	11	0
9	Blida	6	6	0
10	Blida	6	4	2
11	Blida	8	0	8
12	Blida	15	2	13
13	Constantine	8	8	0
14	Constantine	9	6	3
15	Constantine	11	0	11
16	Constantine	6	3	3
17	Constantine	11	5	6
18	Tipaza	8	8	0
19	Tipaza	7	0	7
20	Tipaza	9	5	4
21	Tipaza	7	3	4
22	Bouira	3	3	0
23	Bouira	7	3	4
24	Bouira	2	1	1
25	Médéa	5	3	2
26	Médéa	6	3	3
Total		196	101	95

Table 5. Distribution of *Zymoseptoria tritici* mating types among isolates from the same lesion

Lesion number	Location	N ^a	<i>Mat1-1</i>	<i>Mat1-2</i>
1	Alger	3	1	2
2	Alger	3	3	0
3	Alger	3	1	2
4	Alger	4	4	0
5	Blida	4	4	0
6	Blida	4	1	3
7	Blida	4	0	4
8	Blida	5	1	4
9	Constantine	5	5	0
10	Constantine	4	1	3
11	Constantine	5	5	0
12	Constantine	3	2	1
13	Tipaza	3	3	0
14	Tipaza	3	0	3
15	Tipaza	3	1	2
16	Tipaza	4	2	2
17	Bouira	2	1	1
18	Médéa	4	2	2
Total	...	66	37	29

^a Number of isolates.

Screening for the G143A substitution. The presence of the G143A substitution conferring resistance in *Z. tritici* to QoI-based fungicides was investigated at six locations in Algeria by screening 213 isolates for the G143A mutation. We found six isolates from two different fields (Alger and Constantine) with a single 302-bp amplicon corresponding to the resistance allele A143, whereas the remaining isolates showed the 639-bp amplicon that is indicative of strobilurin sensitivity (Fig. 3). The G143A substitution occurred with a frequency of 0.02 among the locations tested and was found in field 1 in Alger with a frequency of 0.05 (3 of 54; 1 *Mat1-1* and 2 *Mat1-2*) and with a frequency of 0.06 (3 of 48; 2 *Mat1-1* and 1 *Mat1-2*) in field 2 in Constantine. Thus, in both locations, the G143A mutation occurred independently of the mating type of the *Z. tritici* isolates.

The sensitivity bioassay with azoxystrobin-amended PDA plates was performed with the six isolates carrying the G143A mutation and three wild-type isolates from the same fields. In vitro fungicide sensitivity bioassay results were consistent with the PCR assays, because the six isolates considered resistant to QoI formed colonies on the PDA plates amended with azoxystrobin at 1 ppm (Fig. 4), whereas the three isolates considered sensitive to QoI showed no mycelial growth at the same azoxystrobin concentration.

Discussion

The present study reveals that both *Z. tritici* mating types occur with equal frequency distributions at all scales studied. This suggests regular sexual reproduction cycles under Algerian conditions. Our results accord with previous reports from different countries all over the world (Siah et al. 2010b; Zhan et al. 2002), including countries with very different climatic conditions (Abrinbana et al. 2010; Alliou et al. 2014; Boukef et al. 2012; Elbekali et al. 2012), although the *Z. tritici* teleomorph was never reported in natural fields in Algeria. Evidence of the occurrence of sexual reproduction was pointed out by several authors in the past. Zhan et al. (2003), who analyzed a global collection from different continents, including 52 Algerian isolates, reported a high genotypic diversity, which highlighted a significant role of sexual recombination in shaping the *Z. tritici* population structure. Later, Midini and Hamza (2008) compared three populations from Canada, Tunisia, and Algeria and reported that the latter population showed the highest genetic diversity. Moreover, the assessment of virulence on a differential set that included bread and durum wheat cultivars allowed them to differentiate eight significantly different levels of pathogenicity, with four of those being unique to Algeria. Hence, these reports support our data and likely reflect the pathogens' adaptation potential to either environmental conditions or wheat species diversity in the country, as reported by McDonald and Linde (2002).

At the country level, the mating type ratio did not deviate significantly from the expected 1:1 = *Mat1-1*:*Mat1-2* ratio, which was also reported for field populations in other Algerian regions (Alliou et al. 2014). However, the present study further supports the evidence of the occurrence of sexual recombination by showing that both mating types coexist with equal distributions regardless of climatic conditions, agricultural practices, and host distribution, similar to what was found in the French *Z. tritici* population, where the distribution of the mating types was also independent of geographical diversity (Siah et al. 2010b). Some individual fields—field 2 from Alger and fields 1 and 3 from Constantine—did not match 1:1 distributions for both mating type alleles but, at the location level, these frequencies were equal and, hence, such deviations are considered to result from sampling errors (Abrinbana et al. 2010; Elbekali et al. 2012). In our study, deviations may theoretically also result from an overrepresentation of clones because our data were not clone corrected due to the lower number of isolates per location. Indeed, our results confirmed earlier reports on the co-occurrence of both *Z. tritici* mating types at the leaf and lesion scales—even when the sample size was not large—in all locations sampled (Elbekali et al. 2012; El Chartouni et al. 2012; Linde et al. 2002; Siah et al. 2010b; Zhan et al. 2002). At the leaf scale, 62% of tested leaves were colonized by either *Mat1-1* or *Mat1-2* strains, which is not surprising,

because most lesions are believed to be initiated by a single genotype (McDonald and Martinez 1990). As a consequence, successive leaf infections with pycnidiospores from the same haplotype could occur. Therefore, El Chartouni et al. (2012) suggested that genetic diversity at the leaf scale might be a good assessment of asexual reproduction. At the lesion scale, both mating types occurred in 44% of lesions when more than two isolates were assayed, similar to previous findings in other countries (Elbekali et al. 2012; El Chartouni et al. 2012). Given the known bipolar heterothallic mating system of *Z. tritici* (Kema et al. 1996), coexistence of isolates with the opposite mating type at small spatial scales facilitates mating, enabling rapid adaptation to environmental stresses (Linde et al. 2002).

The first QoI-resistant isolates of *Z. tritici* were detected in the United Kingdom in 2001 (Fraaije et al. 2005) and subsequently in five other European countries (Gisi et al. 2005). In the Maghreb countries, strobilurin deployment was limited compared with Europe, and recent investigations have reported the absence of the G143A substitution in Tunisia and Algeria (Alliou et al. 2013; Boukef et al. 2012; Somai-Jemmali et al. 2014; Stammeler et al. 2012). The same results were initially reported in Morocco from a subset of 134 isolates collected in 2008 (El Bekali et al. 2011). However, the same study revealed, for the first time, the occurrence of QoI-resistant isolates in Morocco from a subset of 96 isolates collected in 2010. Similarly, Taher et al. (2014) detected three QoI-resistant isolates in Tunisia.

The collection of 213 field isolates of *Z. tritici* from six locations in Algeria was screened for QoI resistance and the G143A mutation was identified for the first time and confirmed with a fungicide sensitivity bioassay. Although just six mutants were found among these 213 isolates, the finding is significant because, thus far, all studies also in related fungi such as *Pseudocercospora fijiensis* in banana (Amil et al. 2007; Arango Isaza et al. 2016; Chin et al. 2001; Sierotzki et al. 2000) have shown that, once the mutation is selected, its frequency rapidly increases over time. Thus far, it was only observed in Alger and Constantine and none were found in Blida, Tipaza, Bouira, and Médéa, indicating that the QoI resistance likely only recently emerged in Algeria. However, we expect that it will spread rapidly over larger areas, because wind dispersal of ascospores (Torriani et al. 2009) combined with selection (Gisi et al. 2005) and stress-driven unavoidable paternal mating (Ware 2006) will dramatically increase the frequency of this newly emerged QoI resistance.

Our results showed the presence of QoI resistance in Algeria only in fields that were sprayed yearly with Amistar Pro (Azoxystrobin;

Fig. 3. Mismatch amplification mutation assay to determine the G143A mutation in the cytochrome *b* gene in an Algerian *Zymoseptoria tritici* population. Lanes 1 and 2: sensitive control isolates IPO94269 and IPO323; lanes 3, 4, 6, 7, and 8: sensitive isolates carrying the wild-type allele and showing an amplicon of 639 bp; lanes 5, 9, and 10: resistant isolates with the G143A mutation indicated by the 302-bp amplicon; and lane M, 100-bp DNA ladder.

Fig. 4. Quinone outside inhibitor(QoI) fungicide sensitivity assay. Six *Zymoseptoria tritici* isolates carrying the G143A mutation grow normally on potato dextrose agar (PDA) plates amended with azoxystrobin at 1 ppm, whereas the three wild-type isolates were unable to develop colonies (left panel). PDA control plate amended with 1% methanol (right panel).

Syngenta). Information collected during this study showed that DMIs remain the main fungicide class for STB management in Algeria. Strobilurins are applied once a year, mainly for their greening effect, which contributes to a slight yield increase in some genotypes under water deficiency, as reported by Inagaki et al. (2009). Hence, a unique application each season was apparently enough for strobilurin resistance emergence. However, the low frequency of the G143A substitution reported in this study highlights the difference between European and North African populations of *Z. tritici* and provides a unique opportunity to monitor the expansion of QoI-resistant strains in these and other locations.

Finally, the coexistence of the *Z. tritici* mating types across several spatial scales in Algeria opens up many possibilities for further studies, such as sexual crossings between isolates with known or unknown mating types to further the understanding of the epidemiology of *Z. tritici* in a wheat production system that is dominated by durum wheat under Mediterranean conditions.

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