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Frédéric Angelier, Louise Prouteau, François Brischoux, Olivier Chastel, Marie-Hélène Devier, et al.. High contamination of a sentinel vertebrate species by azoles in vineyards: a study of common blackbirds (*Turdus merula*) in multiple habitats in western France. *Environmental Pollution*, 2023, 316, pp.120655. 10.1016/j.envpol.2022.120655 . hal-03907849

HAL Id: hal-03907849

<https://hal.science/hal-03907849v1>

Submitted on 23 Feb 2024

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1 **High contamination of a sentinel vertebrate species by azoles in vineyards: a**
2 **study of Common blackbirds (*Turdus merula*) in multiple habitats in Western**
3 **France**

4

5 Frédéric Angelier^{1,*}, Louise Prouteau^{1,2}, François Brischoux¹, Olivier Chastel¹, Marie-Hélène Devier²,
6 Karyn Le Menach², Stéphan Martin², Bertille Mohring^{1,3}, Patrick Pardon², Hélène Budzinski²

7

8 ¹*Centre d'Etudes Biologiques de Chizé, CNRS-LRU, UMR 7372, Villiers en Bois 79360, France*

9 ²*Université de Bordeaux, CNRS, EPOC, EPHE, UMR 5805, F-33600 Pessac, France*

10 ³*Environmental and Marine Biology, Åbo Akademi University, FI-20250 Turku, Finland*

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17 To be submitted to ***Environmental Pollution***

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19 ***Contact for correspondence:** Frédéric Angelier, frederic.angelier@cebc.cnrs.fr

20

21 **Abstract**

22 Azoles represent the most used family of organic fungicides worldwide and they are used in
23 agriculture to circumvent the detrimental impact of fungi on yields. Although it is known that these
24 triazoles can contaminate the air, the soil, and the water, field data are currently and dramatically
25 lacking to assess if, and to what extent, the use of triazoles could contaminate non-target wild
26 vertebrate species, notably in agroecosystems. In this study, we aimed to document for the first time
27 the degree of blood contamination of a generalist wild bird species by multiple azoles which are used
28 for plant protection and fungi pest control in various habitats. We deployed passive air samplers and
29 captured 118 Common blackbirds (*Turdus merula*) in an agroecosystem (vineyard), a protected
30 forest, and a city in western France. We collected blood and analyzed the plasma levels of 13
31 triazoles and 2 imidazoles. We found that a significant percentage of blackbirds living in vineyards
32 have extremely high plasma levels of multiple azoles (means (pg.g⁻¹); tebuconazole: 149.23,
33 difenoconazole: 44.27, fenbuconazole: 239.38, tetraconazole: 1194.16), while contamination was
34 very limited in the blackbirds from the protected forest and absent in urban blackbirds. Interestingly,
35 we also report that the contamination of blackbirds living in vineyard was especially high at the end
36 of Spring and the beginning of Summer and this matches perfectly with the results from the passive
37 air samplers (i.e., high levels of azoles in the air of vineyards during June and July). However, we did
38 not find any correlation between the levels of plasma contamination by azoles and two simple
39 integrative biomarkers of health (feather density and body condition) in this sentinel species. Future
40 experimental studies are now needed to assess the potential sub-lethal effects of such levels of
41 contamination on the physiology of non-target vertebrate species.

42 **Keywords:** tebuconazole; difenoconazole; fenbuconazole; tetraconazole; agriculture; wildlife

43

44 **Introduction**

45 Over the last century, agricultural practices have intensified to an unprecedented extent in order to
46 increase the yields and the economic value of agriculture (Tilman et al. 2002; Lanz et al. 2018). This
47 intensification has been associated with drastic changes of agricultural landscapes (Gardner 1996),
48 and more specifically with the destruction of many ecological infrastructures (hedgerows, small
49 woods), and with a significant increase in the size of land plots to allow the mechanization of
50 agriculture (e.g., plough, seeder, combine harvester, etc.). As a result of this landscape simplification,
51 monocultures have expanded and agricultural areas are now often characterized by a few very large
52 plots dedicated to the production of a limited numbers of crops (Benton et al. 2003). Such
53 monoculture has led to the surge of pest density and crop diseases (Wilby and Thomas 2002), and
54 thus, to the development of the pesticide industry (Atwood and Paisley-Jones 2017).

55 For example, fungicides are now widely used to circumvent the detrimental impact of fungi on yields
56 in a wide variety of harvests, such as cereals, orchards, vineyards, and even ornamental flowers
57 (Gianessi and Reigner 2006; Moore et al. 2020). Among fungicides, azoles represent the most widely
58 used family of organic fungicides and they have been used for decades in agricultural landscapes
59 (Büchel 1986). Their use was also democratized at the end of the 20th century in private gardens and
60 urban parks to deal with fungi attacks on ornamental flowers, shrubs and trees, and elevated levels
61 of azole compounds can be found in cities (Désert et al. 2018; Schoustra et al. 2019; Zubrod et al.
62 2019). Azoles are antifungal agents that act by inhibiting cell membrane ergosterol synthesis, thereby
63 leading to increased cell permeability and destruction (Vanden Bossche 1985). This mode of action is
64 currently one of the most effective controls of phytopathogenic fungi and azoles now represent the
65 most used family of organic fungicides in crops, such as vineyards, orchards and cereals (Atwood and
66 Paisley-Jones 2017). The azole family includes numerous molecules (e.g., dozens of triazoles and
67 imidazoles). The use of triazoles in US agriculture has increased more than 4-fold during the last
68 decade (Toda et al. 2021). Although the use of some azoles has decreased since the 1980's (ex:

69 triadimefon), the use of others has dramatically increased (ex: tebuconazole, propiconazole) and new
70 triazoles have even started to be used during the last decade (ex: difenoconazole, metconazole, Toda
71 et al. 2021).

72 Azoles are mostly used to circumvent fungi attacks on crops but there is increasing evidence that
73 they can be transferred to the environment. Indeed, recent studies have shown that they can be
74 found in the tissues of humans (e.g. hair, Schummer et al. 2012; Fustinoni et al. 2014; Polledri et al.
75 2018). Triazoles can be transported into the environment through multiple mechanisms of transfer
76 such as volatilization, infiltration and absorption (Mottes et al. 2014; Zhang et al. 2018). As a
77 consequence, the occurrence of azoles and their residues has been reported in several
78 environmental matrices such as soil (Pelosi et al. 2021), water (Ccanccapa et al. 2016; Guarda et al.
79 2020) or air (Coscollà et al. 2010; Désert et al. 2018). In addition, other studies have reported that
80 triazoles can be found in invertebrate species (e.g., Daniele et al., 2018; Pelosi et al. 2021) and in
81 seeds (e.g., Lopez-Antia et al. 2016; Fernandez-Vizcaino et al. 2022), suggesting that these molecules
82 might also be transferred to wildlife through trophic webs (Tassin de Montaigu et al. 2020; Bellot et
83 al. 2022a). Indeed, recent studies have shown that human food could be contaminated by triazoles
84 (Zambonin et al. 2002; Cui et al. 2021). However, field data are currently lacking to assess if, and to
85 what extent, the use of triazoles in agroecosystems and urban areas could contaminate non-target
86 wild vertebrate species (e.g. Bro et al. 2015; Mateo et al. 2016; Esther et al. 2021), and these crucial
87 data are lacking to better understand the threat that these contaminants may represent for wildlife.

88 Some experimental studies have shown that azoles can affect physiological functions and have
89 detrimental effects on fitness in animal models (Cao et al. 2019; Souders et al. 2019; Sun et al. 2020; ;
90 Bellot et al. 2022). A few studies have also demonstrated that the consumption of triazole-coated
91 seeds may impair reproduction and physiological functions of granivorous birds living in
92 agroecosystems (Fernandez-Vizcaino et al. 2020; Lopez-Antia et al. 2021). For example, tebuconazole
93 is known to affect metabolism in passerine birds with a potential impact on body condition and

94 energy regulation (Bellot et al. 2022b). However, we currently lack field data to assess the
95 contamination of wild vertebrates by multiple triazoles in several habitats. Such data are crucial not
96 only to better understand which triazoles are the most likely to contaminate wild vertebrates, but
97 also to determine the degree of contamination of wild vertebrates by these triazoles. These basic
98 data are required to assess the potential threat that different triazoles may entail for wildlife, and by
99 extension for humans (Richard et al. 2021).

100

101 In this study, we aimed to document for the first time the degree of contamination of a generalist
102 wild bird species by multiple azoles that are used for plant protection and fungi pest control in
103 multiple habitats. We captured 118 Common blackbirds (*Turdus merula*) in an agroecosystem
104 (vineyard), a protected forest, and a middle-sized city in western France. We collected blood and
105 analyzed the plasma levels of 13 triazoles and 2 imidazoles by using an on-line SPE coupled to LC-MS
106 methodology. Plasma can be collected ethically and it is a relevant matrix to analyse the levels of
107 azoles by LC-MS/MS because these fungicides are distributed to tissues through the circulatory
108 system (Gross et al., 2020). In addition, we used passive air samplers and available environmental
109 data to evaluate the use of azoles in each habitat (i.e. from a national database on the sales of
110 pesticides (BNVD), City Council, and National Forestry Agency). The Common blackbird is a
111 ubiquitous species, being found in a wide variety of habitats. It is omnivorous, feeds on
112 invertebrates, seeds and berries, and can be found in all three targeted habitats, which allows some
113 comparison (Mohring et al. 2021). Overall, this species is less present in vineyards relative to urban
114 and forest areas, suggesting that it may be impacted by agricultural practices (Mohring et al. 2021).
115 Accordingly, the blackbird has previously been considered as a relevant sentinel species of its
116 environment and used to monitor the degree of contamination of its habitat (Fritsch et al. 2012;
117 Meillère et al. 2016; Diez-Fernandez et al. 2022). To further examine the potential impact of triazoles
118 on this sentinel species, we also focused on two integrative biomarkers of health status (body

119 condition and plumage quality). These biomarkers do not systematically respond to exposure to
120 pesticides (e.g., Lopez-Antia et al. 2018) and additional physiological measurements are necessary to
121 fully evaluate the impact of pesticides on non-target vertebrate species. However, they are useful to
122 consider because they can provide initial warning signs on the potential disrupting effect of
123 pesticides on physiology (metabolism, endocrine axes). We predicted that (1) the contamination of
124 blackbirds by triazoles will be especially important in the vineyards, where triazoles are massively
125 used; (2) the contamination will be moderate in the urban habitat where triazoles could be used by
126 people in private gardens; (3) the contamination will be minimal in the protected forest where
127 triazoles are not used. Importantly, we predicted that (4) different compounds may contaminate
128 blackbirds in each habitat because some triazoles are used for specific crops or purposes only (e.g.
129 difenoconazole in vineyards or tebuconazole in urban areas, Désert et al. 2018). Finally, we also
130 predicted that (5) body condition and feather quality may differ between the three habitats because
131 of contrasted environmental condition and/or agricultural practices; (6) body condition and feather
132 may be correlated with the degree of contamination by specific triazoles if these fungicides have
133 important toxic and sublethal effects on non-target bird species.

134 **Materials and methods**

135 *Study sites*

136 All birds were captured in 2016 and 2019 during the breeding season (April-July) in three
137 geographical areas, which are located in the 'Nouvelle Aquitaine' region in western France. These
138 three areas consist of forest, urban and vineyard areas and they are therefore characterized by
139 different anthropogenic activities and by contrasted use of azoles. Firstly, birds were captured in a
140 protected fenced forest where human activity is extremely limited. This forest covers 2500 ha and is
141 surrounded by arable land. There has been absolutely no use of any azole in this protected forest
142 since 2001 (National Forestry Agency (ONF), personal communication) but specific azoles are used in
143 the arable land surrounding this forest and some birds were captured within 200 m of the edge of

144 the forest (see Annex 2). Secondly, birds were captured in the city of Niort, which is a medium-sized
145 town with multiple parks and gardens. Birds were captured in recreational areas, such as parks or
146 private properties. These parks and gardens were characterized by ornamental trees, shrubs and
147 flowers. Although azoles have not been used by the city since 2014 (Niort City Council, personal
148 communication), inhabitants could use specific azoles in their private backyards. The urban capture
149 sites were located at least 1 km from any agricultural area. Thirdly, birds were captured in the
150 Cognac vineyard where azoles are massively used to protect the vines and the grapes against fungi
151 such as mildew or black rot. To evaluate the use of pesticides in this area through the study period
152 (from 2016 to 2019), we relied on the data from the national database on the sales of pesticides
153 (BNVD, <https://geo.data.gouv.fr/>), which provides the quantity of azoles that are sold each year in
154 each municipality (see Annex 2). Common blackbirds were captured in all three habitats in 2016
155 while they were only captured in the vineyards and the forest in 2019. We did not conduct any
156 capture sessions in 2018 and the field work had to be cancelled/stopped in 2017 because the capture
157 rate was unexpectedly very low. A total of 118 blackbirds were captured in 2016 (urban: 22, forest:
158 28, vineyard: 28) and 2019 (forest:6, vineyard: 34).

159

160 *Study species, capture, measurements and blood sampling*

161 In this study, we focused on the Common Blackbird, a common passerine that is often used as an
162 avian sentinel species in ecotoxicological studies (Fritsch et al. 2012; Meillère et al. 2016; Diez-
163 Fernandez et al. 2022). This species is relatively abundant and can be easily found and captured in
164 forests, cities and vineyards and it is therefore a relevant bird species to study our question of
165 interest (Mohring et al. 2021). Birds were captured by using mist-nets that were located between
166 bushes, trees, or along hedgerows in the forest, urban parks or vineyards. Upon capture, the age of
167 all individuals and the sex of adults were determined by using plumage characteristics. Immediately
168 after capture, a blood sample was collected by puncture of the brachial vein with a 25G needle, and

169 the two inner tail feathers were plucked for feather quality analyses (Meillère et al. 2017). The blood
170 (~100 μ L) was then centrifuged and the plasma was separated from the red blood cells. Plasma was
171 stored at -20 °C until analyses at the LPTC-EPOC (see below). All individuals were measured (wing
172 size) and weighed (body mass) by using a ruler (+/- 0.5 mm) and an electronic scale (+/-0.1 g),
173 respectively. Body condition was then expressed as the residuals of the regression between body
174 mass and wing size (linear regression: $F_{1,116} = 4.05$, $p = 0.046$, $r = 0.184$). All individuals were released
175 within 15 minutes of capture. Feathers were dried in an oven for 48 hours and were then measured
176 with a caliper (total length in mm, ± 0.01 mm) and weighed with a precision scale (± 0.1 mg). A proxy
177 of feather density was calculated by dividing feather mass by feather total length (Meillère et al.
178 2017).

179 *Passive air samplers*

180 In addition to the information on the use of pesticides (ONF, Niort City Council, BNVD database),
181 passive air samplers were deployed in 2017 from the beginning of January to the end of July to with
182 the aim of gaining a better understanding of how azoles were used in the study sites. They house a
183 polyurethane foam (PUF) disk (Tish Environmental, Cleaves, United States), which is uniformly porous
184 to allow chemicals to penetrate. Before application in the field, they were pre-cleaned using an
185 Accelerated Solvent Extractor (ASE Dionex 350, Thermo Fisher Scientific, Asnières sur Seine, France)
186 with dichloromethane solvent (pressure 100 bars; temperature 90 °C; heat 5 min; static 8 min; flush
187 volume 50 %; purge 300 s; 2 cycles), dried and stored in sealed bags.

188 To avoid any potential effect of rain, sunlight, wind or particle deposition and to standardize the
189 sampling process, each PUF disk (14 cm diameter; 1.35 cm thickness; 365 cm² surface area) was
190 deployed in a conventional 'flying saucer' housing that was fixed on a pole at a height of 1.30 m
191 (Tuduri et al. 2006; Estellano et al. 2012). A passive air sampler was deployed in the vineyards while
192 another one was deployed in the protected forest. No passive air sampler was deployed in the urban
193 area because we did not have access to any private properties and such systems had previously been

194 stolen in public urban areas. Passive air samplers were not deployed in 2019 because we did not
195 have the authorization to deploy such systems in any private properties in the urban or vineyard
196 areas during this specific year. Each PUF was left in the field for approximately a month. After each
197 month, the PUF was removed from the device, stored in aluminum foil and in a Ziploc freezer bag
198 and kept at -20 °C until further analysis at the LPTC-EPOC.

199

200 *Plasma levels of azoles*

201 Plasma levels of azoles were determined using LC-MS/MS methodology. Briefly, samples were first
202 extracted using the following procedure: a 1.5 mL microtube, 25 µL of plasma samples, and 30 µL of
203 internal standards solution prepared at 1 ng.g⁻¹ in acetonitrile, were weighed. After a centrifugation
204 step to precipitate proteins, ultra-pure water was added to the supernatant. Then, 600 µL of each
205 extract was injected in the analytical system composed of a Poroshell 120 SB C18 guard column
206 (Agilent) used as on-line SPE cartridge. They were loaded at 2 min at a 1000 µL.min⁻¹ rate with Milli-Q
207 water as eluent. Target compound separation was achieved on a Poroshell Phenyl-Hexyl column
208 (Agilent) using a temperature of 35 °C and a flow rate of 0.3 mL.min⁻¹. Mobile phase A consisted of
209 water with 5 ammonium acetate plus 0.1 % formic acid and mobile phase B of methanol plus 0.1 %
210 formic acid. The following gradient program was applied: 0 to 100 % B over 18 mins, holding at 100 %
211 B for 0.5 mins and returning to initial conditions at 19 mins for 3 mins to even up. The quantification
212 of azoles was performed by using the 6490 Triple Quad mass spectrometer (Agilent) in positive
213 electrospray mode (ESI+) in dynamic multiple reactions monitoring (dMRM). Finally, we checked the
214 analytical sequence with quality controls and we used internal standards to calculate the
215 concentration. The limit of quantification (LOQ) of azoles differed between compounds and varied
216 between 10 and 400 pg.g⁻¹ of plasma except for triadimenol (1500 pg.g⁻¹ of plasma).

217

218 *PUF levels of azoles*

219 A disk quarter was weighed and coiled in stainless-steel 34 mL pre-conditioned cells (Thermo Fisher
220 Scientific, Asnières sur Seine, France). Into the cells, 20 μL of internal standards solution were also
221 added at $0.5 \mu\text{g.g}^{-1}$ in methanol. The conditioning and extraction programs were the same as
222 previously described using acetonitrile instead of dichloromethane solvent. 45 mL of extracts were
223 recovered and evaporated to 3 mL with RapidVap (Labconco, Illkirch, France). The 3 mL were then
224 evaporated to 300 μL under a gentle nitrogen stream. The extracts were then refrigerated until
225 quantification. Quantification was performed by injecting 5 μL of the extracts into a 6495 or 6495B
226 LC-MS/MS (Agilent) with the same separation conditions previously described for the plasma.
227 Concentrations were calculated in ng.PUF^{-1} . LOQ were established taking the maximum extrapolation
228 between LOQ calculated for sample concentration or spiked PUF at 50 ng.PUF^{-1} (see Appendix 1).
229 Extraction and quantification recoveries were also obtained for the spiked PUF (see Appendix 1).
230 Concentrations in ng.PUF^{-1} were then converted in order to estimate the air concentrations (ng.m^{-3})
231 by using estimated air sampling rate for the PUFs ($\text{m}^3.\text{day}^{-1}$) and the duration of PUF deployment in
232 the field. Classically, these rates range from 1 to $8 \text{ m}^3.\text{day}^{-1}$ (Chaemfa et al., 2008, Pozo et al., 2006)
233 and the mean of these two values ($4.5 \text{ m}^3.\text{day}^{-1}$) was therefore used. The average duration of PUF
234 deployment (i.e., 35 days) was chosen to calculate the estimated LOQ in ng.m^{-3} .

235

236 *Statistical analyses*

237 All statistical analyses were run using SAS University edition. Firstly, descriptive and basic statistics
238 were provided for the concentrations of azoles that were quantified in the air and the plasma of
239 blackbirds in the vineyard and the forest habitats. Secondly, Chi-squared tests were used to compare
240 the probability of quantifying specific azoles in the plasma of blackbirds between habitats (forest,
241 urban, and vineyard). General linear models (GLMs) were also run to test the influence of the habitat
242 (urban, forest, and vineyard), the year of sampling (2016, 2019), the date of sampling, and the status
243 (juvenile, adult male, adult female) on the probability of detecting specific azoles in the plasma of

244 blackbirds (the results are detailed in the Appendix 2). These additional GLMs were only run for the
245 four azoles that were detected in a sufficient number of blackbirds (> 15 % of all sampled blackbirds,
246 see Appendix 2). Thirdly, GLMs were used to test the influence of the date of sampling, the year of
247 sampling (2016 and 2019), and the status of the bird (juvenile, adult male, adult female) on the
248 plasma levels of azoles. These analyses were run in the vineyard habitat only and for the four azoles
249 that were quantified in at least 15 % of the sampled blackbirds. They were not run for the urban and
250 the forest habitats because azoles were not quantified in a sufficient number of blackbirds to allow
251 such analyses. Fourthly, GLMs were used to test the influence of the habitat (urban, forest, vineyard),
252 the date of sampling, the year of sampling, and the status of the birds on body condition, and feather
253 density. Finally, GLMs were used to separately test the influence of four azoles (difenoconazole,
254 fenbuconazole, tebuconazole, tetraconazole) on body condition, and feather density (independent
255 variables: year of sampling, date of sampling, habitat, specific plasma azole levels). These analyses
256 were run in the vineyard habitat only and for the four azoles that were quantified in at least 15 % of
257 the sampled blackbirds living in vineyards. They were not run for the urban and the forest habitats
258 because azoles were not quantified in a sufficient number of blackbirds to allow such analyses. All
259 plasma levels of azoles were log transformed to improve the distribution and the variance of the
260 residuals.

261

262 **Results**

263 *Description of the levels of azoles: Passive air samplers*

264 Several triazoles were quantified in the passive air samplers that were deployed in the forest and the
265 vineyard areas (cyproconazole, epoxiconazole, propiconazole, metconazole, tebuconazole,
266 tetraconazole, difenoconazole, penconazole, fenbuconazole), but interestingly, three of them could
267 only be quantified in the vineyard habitat (difenoconazole, penconazole, fenbuconazole) and were
268 totally absent from the forest habitat (Table 1). These quantified azoles correspond overall to the

269 azoles that were sold in 2017 in the vineyard area and in the areas surrounding the forest site (BNVD
270 database, see Annex 2). Importantly, the same azoles were sold from 2016 to 2019 in these areas
271 despite some slight inter-annual variations.

272 In both habitats, azoles were not quantified until April (except tebuconazole, which was quantified at
273 very low levels in March/April in the forest habitat). From April to June, most azoles were similarly
274 quantified in the forest and the vineyard habitat at relatively low (cyproconazole, metconazole,
275 propiconazole: $< 0.03 \text{ ng.m}^{-3}$) or intermediate (epoxiconazole: $< 0.06 \text{ ng.m}^{-3}$) concentrations (Table
276 1). However, in May and to a greater extent in June, five azoles were quantified at high
277 concentrations (difenoconazole, penconazole: $> 0.20 \text{ ng.m}^{-3}$ in June) or very high concentrations
278 (fenbuconazole, tebuconazole, tetraconazole: $> 0.60 \text{ ng.m}^{-3}$ in June) in the vineyard while they were
279 not quantified (difenoconazole, fenbuconazole, and tetraconazole) or only at low concentrations
280 (tebuconazole: $< 0.08 \text{ ng.m}^{-3}$ in June) in the forest habitat (Table 1; Fig. 1).

281

282 *Description of the levels of azoles: Blackbird plasma samples*

283 Several azoles were found in blackbird plasma samples (cyproconazole, difenoconazole,
284 fenbuconazole, flusilazole, metconazole, tebuconazole, tetraconazole) although others were not
285 quantified, notably epoxiconazole, flutriafol, imazalil, penconazole, prochloraz, propiconazole,
286 prothioconazole, tradimefon, and triadimenol (Table 2). No azoles were quantified in all sampled
287 urban blackbirds (Table 2), while several azoles were quantified in vineyard birds, sometimes at very
288 high concentrations, and in most, if not all, captured blackbirds (e.g., tetraconazole, Table 2). In
289 forest birds, a few azoles were quantified in a small percentage of the captured blackbirds, but often
290 at low concentrations (e.g., cyproconazole, tebuconazole, Table 2).

291 Difenoconazole, fenbuconazole, tebuconazole, and tetraconazole were more frequently quantified in
292 blackbirds from vineyards relative to those from the protected forest (difenoconazole: $p = 0.001$,
293 fenbuconazole: $p = 0.013$, tebuconazole: $p = 0.027$, tetraconazole: $p < 0.001$) while this was not the

294 case for cyproconazole, flusilazole, and metconazole (cyproconazole: $p = 0.939$, flusilazole: $p = 0.456$,
295 metconazole: $p = 0.826$). Similarly, difenoconazole, fenbuconazole, tebuconazole, and tetraconazole
296 were more frequently quantified in blackbirds from vineyards relative to those from the urban area
297 (difenoconazole: $p = 0.008$, fenbuconazole: $p = 0.045$, tebuconazole: $p = 0.002$, tetraconazole: $p <$
298 0.001) while this was not the case for cyproconazole, flusilazole, and metconazole (cyproconazole: p
299 $= 0.394$, flusilazole: $p = 0.549$, metconazole: $p = 0.293$). Finally, there was no significant difference in
300 the quantification of any azole between the blackbirds from the protected forest and those from the
301 urban area (all $p > 0.05$).

302

303 *Plasma levels of azoles in vineyard blackbirds*

304 In vineyards, 4 azoles were found in a large proportion of birds and were further analyzed
305 (difenoconazole, fenbuconazole, tebuconazole and tetraconazole). All the others were not quantified
306 in a sufficient percentage of birds to allow further statistical analyses ($< 15\%$ of sampled birds, Table
307 2).

308 Difenoconazole, fenbuconazole, and tetraconazole levels were significantly affected by the date of
309 sampling with increasing difenoconazole levels throughout the season (difenoconazole: $F_{1,57} = 5.30$, p
310 $= 0.025$, fenbuconazole: $F_{1,59} = 9.71$, $p = 0.003$, tetraconazole: $F_{1,60} = 28.26$, $p < 0.001$; Fig. 2) but this
311 relationship was not significant for tebuconazole levels ($F_{1,59} = 2.10$, $p = 0.153$, Fig. 2). In addition,
312 difenoconazole, fenbuconazole, tebuconazole levels were significantly higher in 2016 relative to 2019
313 (difenoconazole: $F_{1,57} = 5.13$, $p = 0.027$, fenbuconazole: $F_{1,59} = 7.68$, $p = 0.007$, tebuconazole: $F_{1,60} =$
314 17.05 , $p = 0.001$, Fig. 2) but this difference was not significant for tetraconazole levels ($F_{1,59} = 0.45$, p
315 $= 0.507$, Fig. 2). Finally, difenoconazole, fenbuconazole, tebuconazole, and tetraconazole levels were
316 not significantly affected by the status of the birds and they did not differ between juveniles, adult
317 males, and adult females (difenoconazole: $F_{2,57} = 2.00$, $p = 0.144$, fenbuconazole: $F_{2,57} = 0.08$, $p =$
318 0.927 , tebuconazole: $F_{2,57} = 0.79$, $p = 0.457$, tetraconazole: $F_{2,57} = 0.48$, $p = 0.624$).

319

320 *Body condition*

321 Body condition was significantly affected by the status of the bird ($F_{2,112} = 15.57, p < 0.001$, Fig. 3A)
322 and the habitat of sampling ($F_{2,112} = 3.79, p = 0.026$, Fig. 3A), but body condition did not differ
323 between 2016 and 2019 ($F_{1,111} = 2.08, p = 0.152$). Body condition also decreased through the
324 breeding season ($F_{1,112} = 4.14, p = 0.044$). Specifically, females were in better condition than males (χ^2
325 = 21.44, $p < 0.001$, Fig. 4A) and juveniles ($\chi^2 = 24.92, p < 0.001$, Fig. 3A), but males and juveniles did
326 not significantly differ in body condition ($\chi^2 = 0.77, p = 0.381$, Fig. 3A). In addition, urban blackbirds
327 were in better condition than vineyard ($\chi^2 = 5.40, p = 0.020$, Fig. 3A) and forest blackbirds ($\chi^2 = 7.06,$
328 $p = 0.008$, Fig. 3A), but vineyard and forest blackbirds did not significantly differ in body condition (χ^2
329 = 0.67, $p = 0.412$, Fig. 3A).

330 In vineyards where four azoles were detected in a sufficient number of blackbirds, we then tested
331 whether body condition was related to the plasma levels of these azoles. In these birds from
332 vineyards, body condition was not correlated with the plasma levels of difenoconazole ($F_{1,56} = 2.70, p$
333 = 0.106), fenbuconazole ($F_{1,56} = 0.20, p = 0.660$), tebuconazole ($F_{1,56} = 0.16, p = 0.687$), and
334 tetraconazole ($F_{1,56} = 1.11, p = 0.296$). However, body condition was significantly affected by the
335 status of the bird ($F_{2,56} = 10.86, p < 0.001$, Fig. 3A) with a higher body condition for females relative to
336 males ($\chi^2 = 16.94, p < 0.001$) and juveniles ($\chi^2 = 14.25, p < 0.001$). Body condition also decreased with
337 the date of sampling ($F_{1,56} = 7.24, p = 0.009$, Fig. 3A), but body condition did not differ between 2016
338 and 2019 ($F_{1,56} = 0.64, p = 0.426$).

339

340 *Feather quality*

341 Feather density was significantly affected by the status of the bird ($F_{2,100} = 7.41, p = 0.001$, Fig. 3B) but
342 not by the habitat of sampling ($F_{2,100} = 0.85, p = 0.429$, Fig. 3B). Feather density did not differ

343 between 2016 and 2019 ($F_{1,100} < 0.01$, $p = 0.948$) but feather density decreased through the breeding
344 season ($F_{1,100} = 5.30$, $p = 0.023$). Specifically, males had denser feathers than females ($\chi^2 = 14.82$, $p <$
345 0.001) but males and juveniles did not significantly differ in feather density ($\chi^2 = 2.79$, $p = 0.095$, Fig.
346 3B). Females and juveniles did not significantly differ in feather density ($\chi^2 = 3.20$, $p = 0.073$, Fig. 3B).

347 In vineyards where four azoles were detected in a sufficient number of blackbirds, we then tested
348 whether body condition was related to the plasma levels of these azoles. Feather density was not
349 correlated with the plasma levels of difenoconazole ($F_{1,51} = 0.93$, $p = 0.338$), fenbuconazole ($F_{1,51} =$
350 0.51 , $p = 0.480$), tebuconazole ($F_{1,51} = 0.19$, $p = 0.662$), and tetraconazole ($F_{1,51} < 0.01$, $p = 0.961$).

351 However, feather density was to all intents and purposes significantly affected by the status of the
352 bird ($F_{2,52} = 2.99$, $p = 0.059$, Fig. 4A) with a higher feather density for males relative to females ($\chi^2 =$
353 5.63 , $p = 0.017$) but no difference between females and juveniles ($\chi^2 = 0.34$, $p = 0.558$) and males and
354 juveniles ($\chi^2 = 2.53$, $p = 0.110$). Feather density was not correlated with the date of sampling ($F_{1,52} =$
355 2.35 , $p = 0.131$), and feather density did not differ between 2016 and 2019 ($F_{1,52} < 0.01$, $p = 0.974$).

356

357 **Discussion**

358 In this study, we examined the plasma levels of multiple azoles of a wild bird species in several
359 habitats. Importantly, we found that blackbirds living in vineyards have high plasma levels of multiple
360 azoles. This high level of contamination was not found in the blackbirds from the protected forest
361 and from the urban habitat. Interestingly, we also report that the contamination of blackbirds living
362 in vineyard was especially great at the end of Spring and the beginning of Summer. This level of
363 contamination by azoles matches perfectly with the results from the passive air samplers that were
364 deployed in the forest and the vineyard habitats in 2017 (i.e., high levels of azoles in the air of
365 vineyards during June and July). However, we did not find any correlation between the levels of
366 plasma contamination by azoles and two simple integrative biomarkers of health (feather density
367 and body condition) in this sentinel species. To better evaluate the impact of such contamination on

368 the health of non-target vertebrate species, experimental studies focusing on key physiological and
369 biochemical mechanisms are now required.

370

371 *Contamination of blackbirds by azole fungicides*

372 We analyzed the plasma of a wild vertebrate sentinel species to detect specific azole fungicides that
373 are used for pest control in multiple habitats. Importantly, we showed that the degree of
374 contamination of wild birds by azole fungicides varies significantly between habitats. Indeed,
375 multiple azoles were found in very high concentration in the plasma of blackbirds living in vineyards.
376 In contrast, and contrary to our prediction, azoles could not be quantified in the plasma of urban
377 blackbirds. Finally, some azoles were found in the plasma of some forest blackbirds but at relatively
378 low concentrations.

379

380 These results demonstrate that azole fungicides can contaminate wild birds living in agroecosystems,
381 and especially in the vineyards that are characterized by a massive use of fungicides in general, and
382 of azoles in particular (Komarek et al. 2010). These findings corroborate recent studies which have
383 reported the presence of azole fungicides in the urine or the hair of humans working in vineyards
384 (Fustinoni et al. 2014; Mercadante et al. 2019; Polledri et al. 2018). Regarding wild vertebrates, data
385 are however very limited. A few studies have found that triazoles could be found in wild vertebrates
386 living in agroecosystems but these studies have only found residues of azole fungicides in a limited
387 number of very specific matrices (e.g., eggs of birds, Bro et al. 2015; Millot et al. 2015; feces of birds,
388 Esther et al. 2021) or in the food that could be consumed by wild vertebrates (e.g., seeds, Fernandez-
389 Vizcaino et al. 2020, 2022; Lopez-Antia et al. 2016; invertebrates, Pelosi et al. 2021). To our
390 knowledge, the level of contamination of wild vertebrates by azole fungicides has never been
391 determined in vineyard ecosystems. Here, we report very high levels of contamination for four
392 specific compounds that could be of serious concern for non-target species (tetraconazole,

393 tebuconazole, difenoconazole, fenbuconazole), and that could contribute to the current declines of
394 farmland birds (Cabodevilla et al. 2021).

395

396 In cities, azole fungicides are mainly used against fungi attacks of ornamental flowers, shrubs and
397 fruit trees (Bertoncini et al. 2012; Muratet and Fontaine 2015) but the absence of azole
398 contamination in urban blackbirds suggests that azole fungicides are used to a very limited extent in
399 cities, or at least in the city where we sampled blackbirds. Our study therefore suggests that urban
400 blackbirds probably do not suffer from any potential detrimental effects of triazoles on physiology or
401 performance (survival or reproduction, see Lopez-Antia et al. 2021 or Bellot et al. 2022b for examples
402 of such effects in birds) although they may be affected by other urban contaminants (e.g., heavy
403 metals, Meillère et al. 2016; Fritsch et al. 2019; Bauerova et al. 2020).

404

405 The contamination of a few blackbirds by azole fungicides in the protected forest was unexpected
406 because of the absence of use of fungicides in that habitat. This astonishing result could be explained
407 by the past use of azole fungicides to protect the harvested wood when the forest was not protected
408 (Reinprecht 2010) but this is highly unlikely because this forest has been protected for almost 20
409 years and the half-life of most azole fungicides is limited to a maximum of a few months. Instead, this
410 result could be explained by the surroundings of the protected forest, which is characterized by
411 cereal crops, in which azole fungicides are used and can contaminate soil and invertebrates (Pelosi et
412 al. 2021). Indeed, the National database on the sale of pesticides (BNVD) confirms that several azoles
413 are used in the crops located around the forest (especially cyproconazole, epoxiconazole,
414 metconazole, propiconazole, tebuconazole, and tetraconazole, see Annex 1). The use of azole
415 fungicides in these crops could potentially contaminate forest blackbirds if the azoles are transferred
416 to the forest through air circulation, as suggested by the results from our passive air samplers that
417 were deployed in the middle of this protected forest. In addition, blackbirds could be contaminated
418 by azoles while foraging on the edges of the protected forest (i.e., in cereal crops), especially if they

419 feed on treated seeds (Fernandez-Vizcaino et al. 2020; Tassin de Montaigu & Goulson 2020).
420 However, it is important to note that azole fungicides were found in only a few birds and at very
421 moderate levels (see Table 2), demonstrating that blackbirds living in this protected forest are not
422 highly contaminated by azole fungicides.

423

424 *Passive air samplers and the contamination of blackbirds by azole fungicides*

425 Our study reports that the degree of contamination of blackbird plasma by azole fungicides mirrors
426 what is released in the environment. Firstly, we found that 4 main azoles were quantified at high
427 concentrations in the PUFs that were deployed in the vineyards, and these 4 same azoles were
428 quantified in a large percentage of vineyard blackbirds. Importantly, these azoles were quantified
429 mostly in June/July, when they were also detected in the plasma of blackbirds. In addition, they were
430 not quantified before June either in the PUFs or in the blackbird plasmas. Finally, three of these four
431 azoles were not detected in the PUFs that were deployed in the protected forest (difenoconazole,
432 fenbuconazole, tetraconazole) and they were also not quantified in the blackbirds living in this forest
433 (or in only a very small percentage of plasma samples). Interestingly, a few other triazoles were
434 quantified in the PUFs and concomitantly in blackbird plasma samples, either in the vineyard or in
435 the protected forest (e.g., metconazole, cyproconazole). For example, tebuconazole was quantified
436 in the PUF that was deployed in the protected forest and this specific triazole was also quantified in
437 some blackbirds living in this forest. All these results demonstrate that the Common Blackbird is an
438 appropriate sentinel species to investigate the release of azole fungicides in the environment.

439

440 Unfortunately, we were not able to deploy a PUF in the urban habitat, precluding us from linking the
441 levels of azoles in the passive air samplers with those that were quantified in urban blackbirds. It is
442 also important to note that the passive air samplers were deployed in 2017 while the sampling of
443 blackbirds was conducted in 2016 and 2019 (unfortunately, our capture rate was very low in 2017 in
444 most habitats). This could explain some of the discrepancies that we reported between passive air

445 samplers and blackbird plasmas (e.g., epoxiconazole that was detected in the PUFs but not in
446 blackbirds). However, it is very unlikely that this affected our interpretation since the same azole
447 fungicides were sold in the vineyard sites where we sampled blackbirds over that period (i.e., from
448 2016 to 2019, BNVD database, see Annex 1). Moreover, the same main azoles were quantified in
449 blackbird plasma in 2016 and 2019 in the vineyards (tetraconazole, tebuconazole, fenbuconazole,
450 difenoconazole) and in the protected forest (tebuconazole), further supporting the data from the
451 BNVD database (see Annex 1), and the idea that the same azoles were used over that period in
452 vineyards.

453

454 Among the 4 main azoles that were quantified in vineyard blackbirds, we found that the levels of
455 azoles in blackbird plasma was much more important for some of them (e.g., tetraconazole) than
456 others (e.g., difenoconazole). This may be related to a higher use or release of specific azoles in the
457 environment but also suggests that some triazoles are more likely to contaminate wild vertebrates
458 than others, or at least more likely to be quantified in wild bird plasma. This could be related to
459 specific chemical properties of specific triazoles (half-life, solubility) and to the ability of the
460 organisms to metabolize these azole fungicides (see Gross et al. 2020). Thus, some azole fungicides
461 may be more prone to contaminate the food web than others (soils, plants, invertebrates, etc.), and
462 as a result, blackbirds may ingest higher quantities of some triazoles relative to others. It is also
463 possible that blackbirds absorb some triazoles to a greater extent than others. Similarly, blackbirds,
464 and wild vertebrates in general, may metabolize some azoles quicker than others (Gross et al. 2020).
465 Complementary studies are definitely needed to better understand the metabolism and the
466 elimination of azoles fungicides in wild vertebrates (see Hao et al. 2021 for an example).

467

468 *Impact of the plasma levels of azoles on body condition and feather density*

469 Despite important differences in plasma levels of azole fungicides between the three habitats, we did
470 not report any strong difference in two proxies of health status between these habitats. Feather

471 density was similar between the vineyard, the urban and the forest habitats. We found that urban
472 birds were in better condition than vineyard blackbirds, but it is very unlikely to be related to the
473 contamination by azoles. Indeed, vineyard blackbirds were of similar body condition as forest
474 blackbirds despite much higher levels of azoles in their plasma. In addition, we did not find any
475 significant relationship between either body condition or feather density and any plasma levels of
476 azoles for the birds living in vineyards, suggesting that the most contaminated birds were not
477 dramatically impacted by this contamination. However, previous studies have found that this specific
478 triazole can impair reproduction in vertebrates (e.g., Lopez-Antia et al. 2013, 2021) and we must
479 remain very cautious because azole fungicides can disappear quite quickly from the plasma after
480 ingestion or inhalation (Gross et al. 2020; Hao et al. 2021). This fast elimination from the plasma
481 could also explain why we did not find strong correlations between plasma levels of azoles and our
482 proxies of health status. The study of complementary and integrative matrices appears relevant to
483 further test the link between the degree of contamination and the health of non-target species (e.g.,
484 feces, Esther et al. 2021 and feather: Rutkowska et al. 2018). Importantly, our study also
485 demonstrates that the plasma levels of azole fungicides are especially high in June/July, which
486 corresponds to the period of reproduction, and therefore, to the period of development for many
487 vertebrate species. This means that chicks and juveniles can endure very high levels of plasma azoles
488 during the ontogenetic phase of their organismal systems, and this is of particular concern because
489 previous studies have found negative impacts of azole fungicides on reproduction and development
490 in vertebrates (e.g., Fernández-Vizcaíno et al. 2020; Lopez-Antia et al. 2021). In this study, we only
491 looked at two simple and integrative biomarkers of health status that are also known to be affected
492 by multiple environmental factors (e.g., food abundance, weather). These simple biomarkers did not
493 necessarily allow us to detect sublethal effects and further studies are needed to test the impact of
494 azole fungicides on several physiological mechanisms such as glucose and protein regulation,
495 mitochondrial function, metabolism, and endocrine mechanisms (see Li et al. 2019; Cao et al. 2019;
496 Souders et al. 2020; Fernandez-Vizcaino et al. 2020; Bellot et al. 2022b for some examples). Finally,

497 our study reports the plasma levels of azoles for birds living in multiple habitats, including highly
498 contaminated ones. These data will be of primary importance for further experimental captive
499 studies because they will allow ecotoxicologists to know which doses of azoles need to be used to
500 test the impact of environmentally realistic doses of azole fungicides on physiological systems and
501 performance of vertebrates.

502

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504

505 **Acknowledgements**

506 This research has been funded by the Agence Nationale de la Recherche (ANR project URBASTRESS,
507 ANR-16-CE02-0004-01 to F.A., and ANR project VITIBIRD to F.A.), by the Région Nouvelle-Aquitaine
508 (MULTISTRESS project), by the Agence Nationale de Sécurité Sanitaire de l'Alimentation, de
509 l'Environnement et du Travail (ANSES project BiodiTox # 2019/1/031), by the Contrat Plan-Etat
510 Région (CPER) ECONAT, and by the Centre National de la Recherche Scientifique. We thank H.
511 Bidault, C. Eraud, M. Lahouarnat, Y. Lechauve, H. Lormée, and A. Villers (Office Français de la
512 Biodiversité, OFB) for their great and invaluable help during field work. We also thank the ONF
513 (Office national des Forêts), the 'Mairie de Niort', C. and J-C. Barbraud, C. and B. Bégau, A. Fuseau,
514 and R. Seguin for giving us access to capture sites. We thank L. Runacre for correcting and improving
515 the English.

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733

734 **Table 1:** Concentrations of azoles in the passive air samplers that were deployed in the vineyard and the forest habitats. PUFs were deployed for
 735 approximately a month before being replaced by a new one. The concentrations of azoles were calculated in ng.m⁻³ after taking into account the duration of
 736 deployment of each PUF and an estimated air sampling rate for the PUFs (4.5 m⁻³.day⁻¹, see Chaemfa et al., 2008).

	Forest						Vineyard							
	Date of PUF deployment	Dec 9	Jan 12	Feb 15	Mar 14	Apr 13	Jun 2	Date of PUF deployment	Dec 9	Jan 12	Feb 15	Mar 14	Apr 13	Jun 2
	Date of PUF retrieval	Jan 12	Feb 15	Mar 14	Apr 13	Jun 2	Jul 7	Date of PUF retrieval	Jan 12	Feb 15	Mar 14	Apr 13	Jun 2	Jul 7
Cyproconazole	< LOQ	< LOQ	< LOQ	< LOQ	0.023	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.026	0.0190	
Difenoconazole	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.240	
Epoxiconazole	< LOQ	< LOQ	< LOQ	< LOQ	0.040	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.058	< LOQ	
Fenbuconazole	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.027	0.870	
Flusilazole	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	
Flutriafol	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	
Imazalil	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	
Metconazole	< LOQ	< LOQ	< LOQ	< LOQ	0.013	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.017	< LOQ	
Penconazole	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.360	
Prochloraz	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	
Propiconazole	< LOQ	< LOQ	< LOQ	< LOQ	0.014	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.013	< LOQ	
Tebuconazole	< LOQ	< LOQ	< LOQ	0.015	0.076	0.025	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.330	0.660	
Tetraconazole	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.290	0.620	
Tradimefon	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	
Triadimenol	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	

737

738

739 **Table 2:** Frequency of quantification and concentrations for multiple azoles in plasma samples of blackbirds from the urban, the forest and the vineyards
740 habitats in 2016 and 2019. Note that the urban habitat was not studied in 2019. * In 2016, the concentration of tetraconazole in vineyard blackbirds was
741 expressed with (regular font) and without (*italic font*) an outlier.

2016												
Urban (n = 22)					Forest (n = 28)				Vineyard (n = 28)			
Concentrations (pg.g ⁻¹ plasma)	Frequency (%)	Range (min-max)	Median	Mean ± SD	Frequency (%)	Range (min-max)	Median	Mean ± SD	Frequency (%)	Range (min-max)	Median	Mean ± SD
Cyproconazole	0%	-	-	-	0%	-	-	-	0%	-	-	-
Difenoconazole	0%	-	-	-	0%	-	-	-	39%	10 - 156.7	24.4	47.9 ± 49.1
Epoxiconazole	0%	-	-	-	0%	-	-	-	0%	-	-	-
Fenbuconazole	0%	-	-	-	0%	-	-	-	29%	33.3 - 945	155.3	287.3 ± 314.3
Flusilazole	0%	-	-	-	0%	-	-	-	0%	-	-	-
Flutriafol	0%	-	-	-	0%	-	-	-	0%	-	-	-
Imazalil	0%	-	-	-	0%	-	-	-	0%	-	-	-
Metconazole	0%	-	-	-	7%	35.3 - 48.6	41.95	42 ± 9.4	4%	34.6 - 34.6	34.6	34.6
Penconazole	0%	-	-	-	0%	-	-	-	0%	-	-	-
Prochloraz	0%	-	-	-	0%	-	-	-	0%	-	-	-
Propiconazole	0%	-	-	-	0%	-	-	-	0%	-	-	-
Tebuconazole	0%	-	-	-	11%	62.7 - 281.3	63	135.7 ± 126.1	54%	36.9 - 521.8	105.5	175.4 ± 151.6
Tetraconazole*	0%	-	-	-	14%	71.5 - 276.8	132.2	153.2 ± 95.6	89%	28.5 - 59976.5 <i>28.5 - 8729.4</i>	356.9 285	3545.2 ± 11910.3 <i>1045.2 ± 1836.8</i>
Tradiméfon	0%	-	-	-	0%	-	-	-	0%	-	-	-
Triadimenol	0%	-	-	-	0%	-	-	-	0%	-	-	-

2019												
Urban (n = 0)					Forest (n = 6)				Vineyard (n = 34)			
Concentrations (pg.g ⁻¹ plasma)	Frequency (%)	Range (min-max)	Median	Mean ± SD	Frequency (%)	Range (min-max)	Median	Mean ± SD	Frequency (%)	Range (min-max)	Median	Mean ± SD
Cyproconazole	-	-	-	-	17%	48.4 - 48.4	48.38	48.4 ± NA	6%	78.2 - 211.6	144.9	144.9 ± 94.4
Difenoconazole	-	-	-	-	0%	-	-	-	15%	18.2 - 85.3	23.27	36.3 ± 28.3
Epoxiconazole	-	-	-	-	0%	-	-	-	0%	-	-	-
Fenbuconazole	-	-	-	-	0%	-	-	-	6%	40.1 - 55.3	47.715	47.7 ± 10.7
Flusilazole	-	-	-	-	0%	-	-	-	3%	16.4 - 16.4	16.35	16.4
Flutriafol	-	-	-	-	0%	-	-	-	0%	-	-	-
Imazalil	-	-	-	-	0%	-	-	-	0%	-	-	-
Metconazole	-	-	-	-	0%	-	-	-	6%	65.2 - 167.4	116.285	116.3 ± 72.3
Penconazole	-	-	-	-	0%	-	-	-	0%	-	-	-
Prochloraz	-	-	-	-	0%	-	-	-	0%	-	-	-
Propiconazole	-	-	-	-	0%	-	-	-	0%	-	-	-
Tebuconazole	-	-	-	-	17%	83.7 - 83.7	83.7	83.7 ± NA	15%	26 - 222.2	36.2	70.7 ± 84.9
Tetraconazole	-	-	-	-	0%	-	-	-	97%	24.3 - 8341.4	332.96	1302.5 ± 2054.2
Tradiméfon	-	-	-	-	0%	-	-	-	0%	-	-	-
Triadimenol	-	-	-	-	0%	-	-	-	0%	-	-	-

742

743 **Figure legends**

744

745 **Figure 1:** Azole air concentrations ($\text{ng}\cdot\text{m}^{-3}$) derived from deployments of PUF disk passive air samplers
746 in the protected forest habitat (green) or in the Cognac vineyard (red) between December 2016 and
747 July 2017. Difenoconazole (A), fenbuconazole (B), tebuconazole (C), and tetraconazole (D) were not
748 quantified in the PUF from December to March-April in both habitats, but their air concentrations
749 increased from April to July in the vineyard habitat.

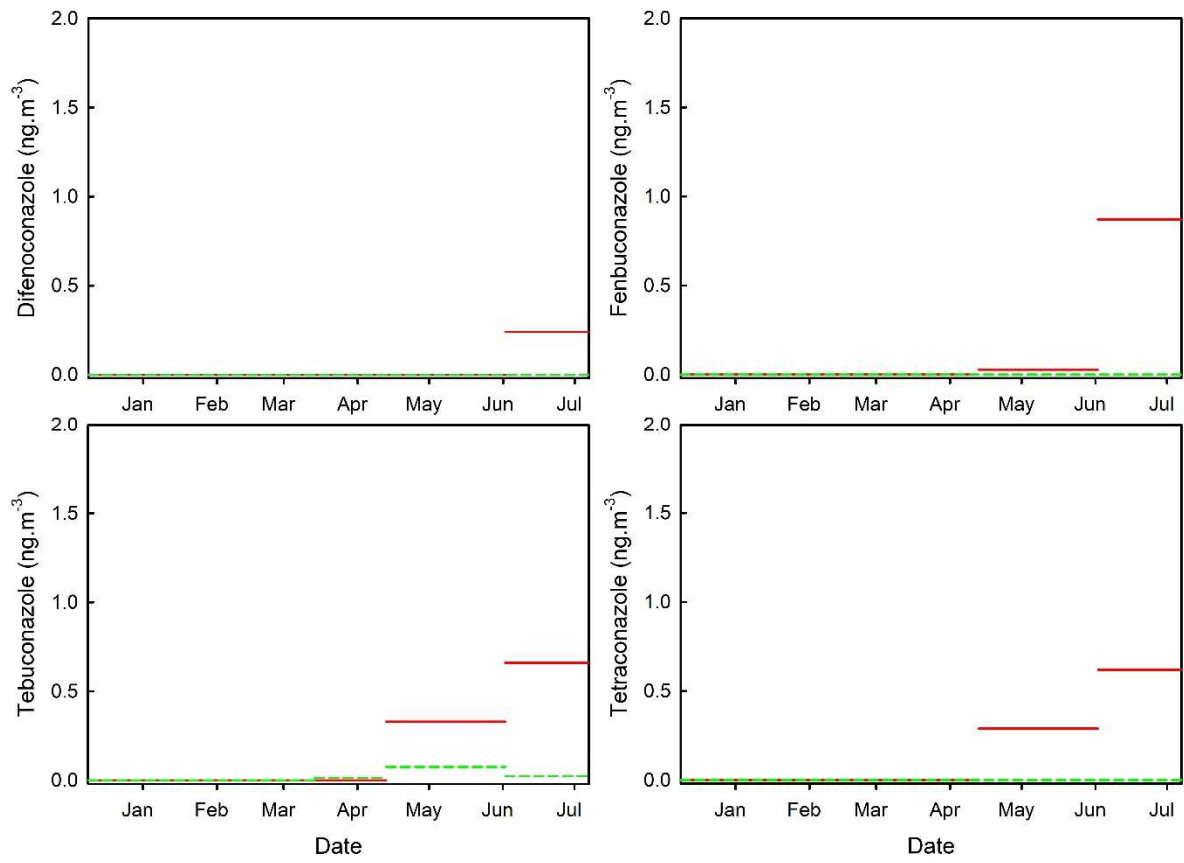
750 **Figure 2:** Plasma levels of the azoles ($\text{pg}\cdot\text{g}^{-1}$ of plasma) that were quantified in at least 15% of the
751 captured birds in the Cognac vineyard (A: difenoconazole, B: fenbuconazole, C: tebuconazole, D:
752 tetraconazole). The birds that were captured in 2016 and 2019 are represented in black and white
753 respectively. Julian date represents the date with the 1st of January being represented by the number
754 1. Plasma levels of difenoconazole, fenbuconazole, and tetraconazole significantly increased through
755 the season. Plasma levels of difenoconazole, fenbuconazole, and tebuconazole were significantly
756 higher in 2016 relative to 2019. Note that the scales are different between the four azoles.

757 **Figure 3:** Influence of age, sex and habitat on body condition (A) and feather density (B) in blackbirds.
758 Urban birds were in better condition than vineyard and forest birds. Adult females (white) were also
759 in better condition than adult males (black) and juveniles (grey). Data are expressed as means \pm
760 standard errors. Different letters represent significant differences between habitats ($p < 0.05$).

761

762

763 **Figure 1:**

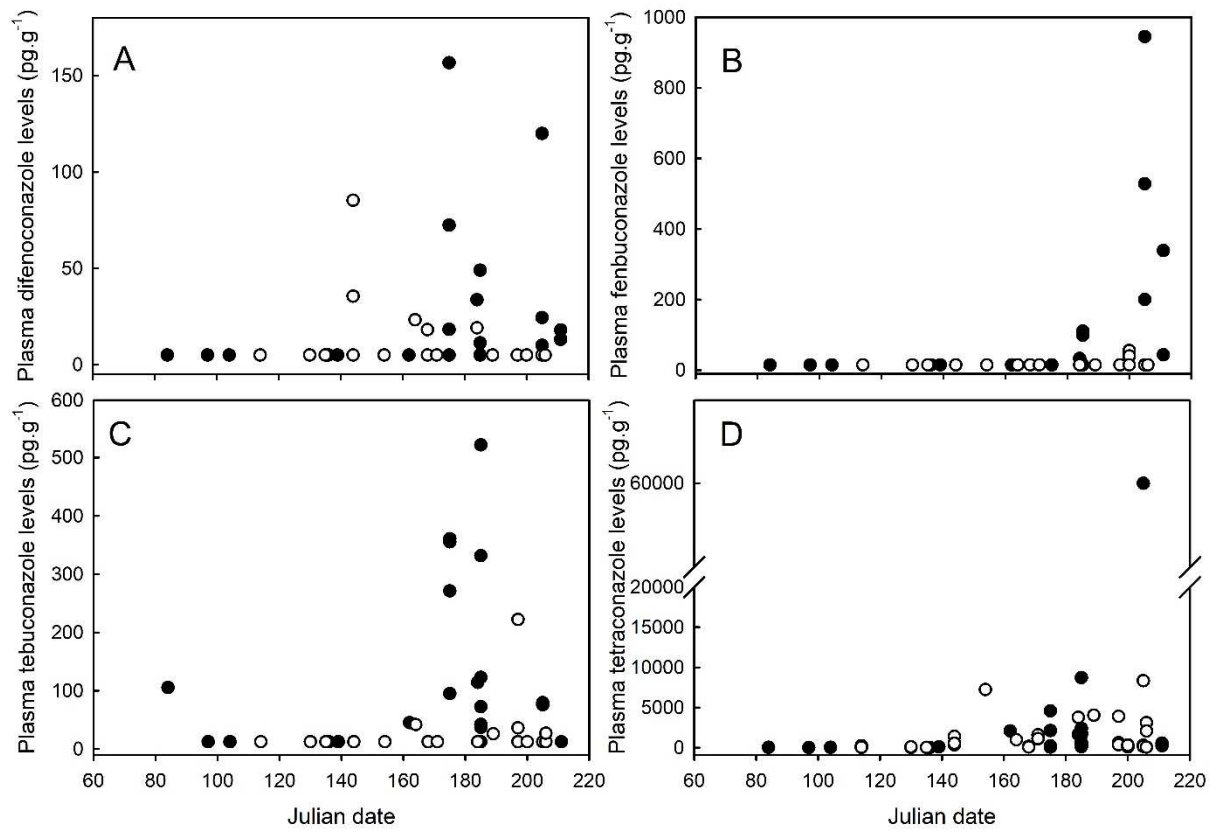


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765

766 **Figure 2:**

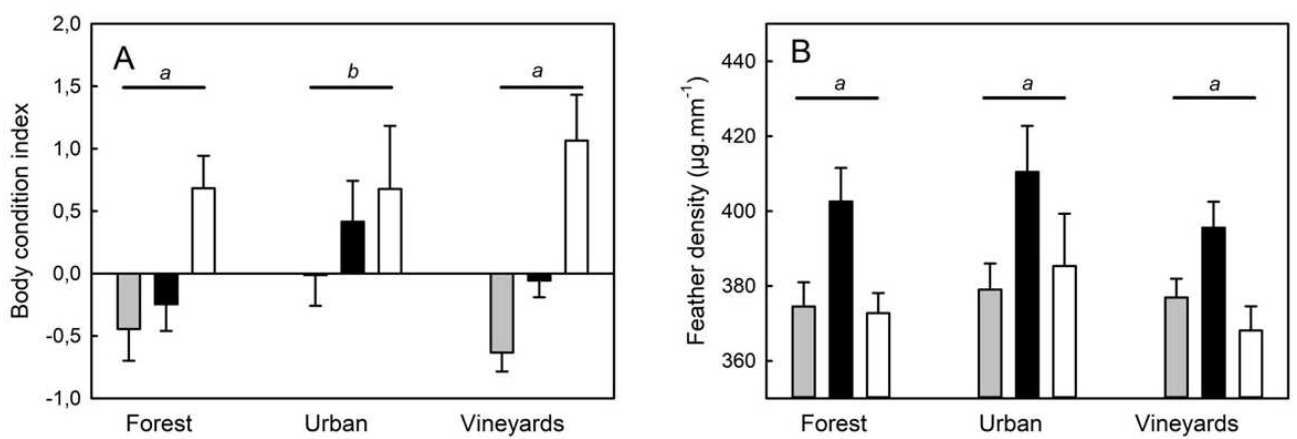
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769 **Figure 3:**

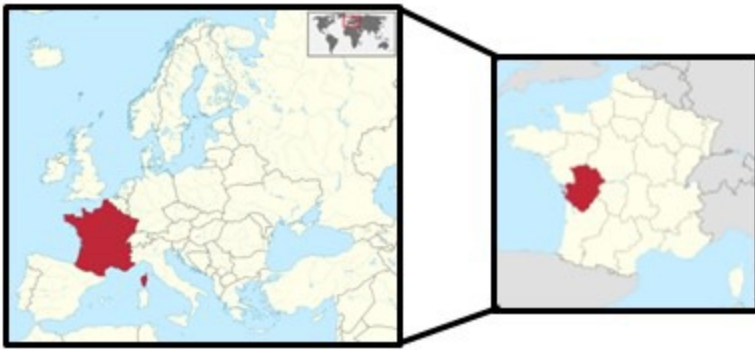
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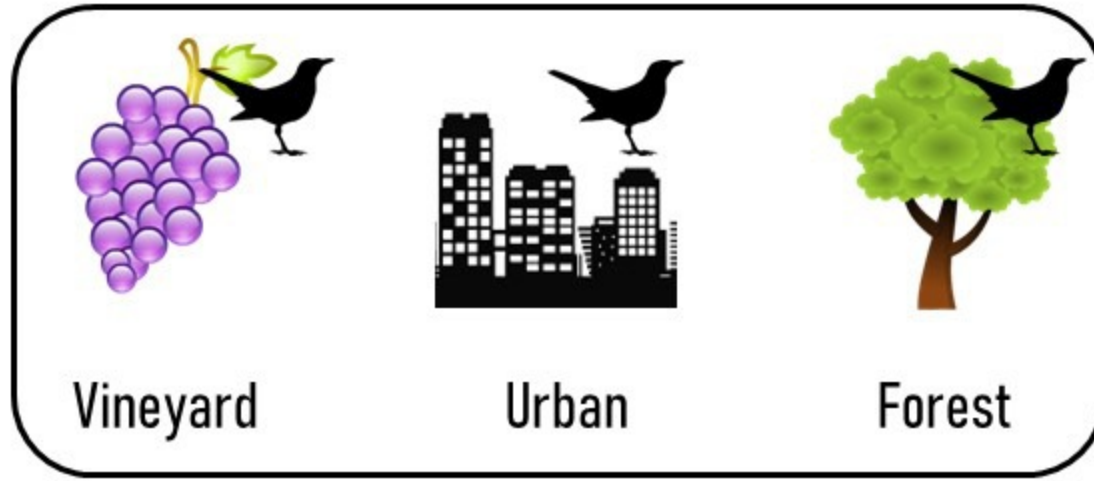
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Western France



Vineyard

Urban

Forest

Contamination by azoles

