1 **Citizen-science surveillance of triazole-resistant *Aspergillus***

2 ***fumigatus* in UK residential garden soils**

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11 Abstract

12 Compost is an ecological niche for *Aspergillus fumigatus* due to its role as a decomposer of organic

13 matter and its ability to survive the high temperatures associated with the composting process.

14 Subsequently, composting facilities are associated with high levels of *A. fumigatus* spores that are

15 aerosolised from compost and cause respiratory illness in workers. In the UK, gardening is an activity

16 enjoyed by individuals of all ages and it is likely that they are being exposed to *A. fumigatus* spores

17 when handling commercial compost or compost they have produced themselves. In this study, 246

18 citizen scientists collected 509 soil samples from locations in their garden in the UK, from which

19 were cultured 5,174 *A. fumigatus* isolates. Of these isolates, 736 (14%) were resistant to

20 tebuconazole: the third most-sprayed triazole fungicide in the UK, which confers cross-resistance to

21 the medical triazoles used to treat *A. fumigatus* lung infections in humans. These isolates were

22 found to contain the common resistance mechanisms in the *A. fumigatus cyp51A* gene TR_{34}/L98H or

23 TR_{46}/Y121F/T289A, and less common resistance mechanisms TR_{34}, TR_{53},

24 TR_{46}/Y121F/T289A/S363P/I364V/G448S and $(TR_{46})^2$/Y121F/M172I/T289A/G448S. Regression

25 analyses found that soil samples containing compost were significantly more likely to grow

26 susceptible and tebuconazole-resistant *A. fumigatus* than those that did not, and that compost

27 samples grew significantly higher numbers of *A. fumigatus* than other samples.

28 Importance

29 These findings highlight compost as a potential health hazard to individuals with pre-disposing

30 factors to *A. fumigatus* lung infections, and a potential health hazard to immunocompetent

31 individuals who could be exposed to sufficiently high numbers of spores to develop infection.

32 Furthermore, this study found that 14% of *A. fumigatus* isolates in garden soils were resistant to an

33 agricultural triazole, which confers cross-resistance to medical triazoles used to treat *A. fumigatus*

34 lung infections. This raises the question of whether compost bags should carry additional health

35 warnings regarding inhalation of *A. fumigatus* spores, whether individuals should be advised to wear

36 facemasks whilst handling compost or whether commercial producers should be responsible for

37 sterilising compost before shipping. The findings support increasing public awareness of the hazard

38 posed by compost and investigating measures that can be taken to reduce the exposure risk.

39 **Introduction**

40 The fungus *Aspergillus fumigatus* plays an important role in the environment as a decomposer,

41 recycling nutrients from decaying plant matter into the soil. This highly sporulating mould is

42 commonly found in woodchip piles, compost from household waste, sewage, sludge and mouldy

43 hay[1], where its thermotolerance enables it to proliferate during the thermogenic phase of

44 composting when temperatures reach 40-60°C[2]. The small size of *A. fumigatus* spores (2-3 µm) and

45 their hydrophobicity means they are easily aerosolised and transported on air currents, making *A.*

46 *fumigatus* a globally ubiquitous fungus[3]. Exposure to this mould is medically important and it is

47 estimated that humans inhale several hundred *A. fumigatus* spores per day[4], which can trigger an

48 immunoinflammatory response resulting in severe asthma with fungal sensitisation (SAFS) or allergic

49 bronchopulmonary aspergillosis (ABPA)[5]. The size of the spores allows them to bypass mucociliary

50 clearance in the lung[6] whereupon they must then evade clearance by the host innate and adaptive

51 immune responses[7]. If they survive, germinated spores establish in lung cavities where they can

52 eventually cause chronic pulmonary aspergillosis (CPA). CPA affects apparently immunocompetent

53 individuals with an existing lung condition such as ABPA, chronic obstructive pulmonary disease

54 (COPD), tuberculosis (TB) or lung cancer, or underlying immune dysfunction due to diabetes,

55 rheumatoid arthritis or alcoholism[8]. If the host immune system is unable to prevent spores from

56 entering the bloodstream then invasive aspergillosis (IA) develops, which is a life-threatening

57 infection associated with ~58% survival[9]. Individuals who are immunocompromised due to

58 treatment with immunosuppressants, chemotherapy or HIV/AIDS infection are at greatest risk of IA

59 [9]. Furthermore, individuals admitted to intensive care units (ICU) with severe influenza infection are

60 at risk of developing influenza-associated pulmonary aspergillosis (IAPA), which is associated with

61 increased mortality[10]. A similar disease is now being observed for COVID-19 associated pulmonary

62 aspergillosis (CAPA) in individuals with severe COVID-19 infection[11]. It was estimated that in the UK

63 in 2011 there were ~178,000 individuals living with ABPA, 3,600 with CPA and 2,900 with IA, plus an

64 additional 377-1,345 cases of IA in critical care patients[12]. The number of patients in the UK

65 presenting with infections that are resistant to one or more of itraconazole (ICZ), voriconazole (VCZ)

66 and posaconazole (PCZ) – the frontline triazole drugs for treating aspergillosis – has risen from 3-7%

67   between 1999 and 2001 to 14-20% between 2007 and 2009[13]. Triazole-resistant infections are

68   associated with treatment failure, salvage therapy with more toxic antifungals and increased case

69   fatality rates (CFR), with CFRs up to 88% reported for triazole-resistant IA[14].

70   Triazole-resistance is most commonly caused by polymorphisms in the *cyp51A* gene, which results in

71   increased production of, or configurational changes in, lanosterol-14a-demethylase; an enzyme

72   involved in ergosterol biosynthesis and the binding target of triazole drugs. An environmental route

73   for the acquisition of triazole-resistant infections has been proposed due to the increase of

74   infections caused by *A. fumigatus* isolates with a tandem repeat (TR) in the promoter region of

75   *cyp51A* coupled with single nucleotide polymorphisms (SNPs) in the coding region leading to amino

76   acid substitutions in the protein, which are frequently recovered from air and soil samples globally[15].

77   This is likely due to the use of fungicides epoxiconazole, tebuconazole, propiconazole,

78   difenoconazole and bromuconazole, which have similar molecular structures to the medical triazoles

79   and show cross-resistance[16]. In 2008, these were the second, third, sixth, ninth and seventeenth

80   most sprayed triazoles in agriculture in the UK, respectively[17]. In agriculture, triazoles are applied to

81   wheat, beans, carrots, oilseed rape, soft fruits and vines; in horticulture, they are used to sterilise

82   bulbs and to control fungal diseases in lawns and ornamentals; and in industry they are used as

83   wood preservatives and antifouling agents in leather, paper, textiles, paints and adhesives[17].

84   The UK government is committed to reducing carbon dioxide emissions by diverting waste from

85   landfill and incineration to composting[18], and compost features in the government's Food 2030

86   strategy for improving the productive capacity of soil[19]. Compost producers accept input material

87   from agriculture, horticulture, forestry, wood and paper processing, leather and textiles industries,

88   household and garden waste, which are highly likely to contain triazole residues. In 2007, 90% of

89   composting facilities in the UK produced compost in open windrows[20]; where organic waste is

90   shredded, mixed and placed in uncovered rows that are turned regularly during the composting

91   process to improve oxygenation of the waste and to distribute heat and moisture. Composting

92   facilities are known to produce large numbers of *A. fumigatus* spores[20–27], with resulting negative

93   health impacts on compost handlers[28–36], and there is evidence from the Netherlands that

94   composting material also produces large numbers of triazole-resistant spores[37,38]. In 2017, UK

95   households spent approximately £450 million on compost[39] and apply it more liberally to their

96   gardens at 300 tonnes per hectare (t/ha) than the 50 t/ha applied to agricultural land[40].

97   Furthermore, more than a third of households with access to a garden report composting their

98   garden and/or kitchen waste[41]. This means that a substantial proportion of the UK population is

99   handling compost on a regular basis, with potential exposure to high levels of *A. fumigatus* spores

100   that may have developed triazole-resistance from composts that contain triazole residues. Indeed,

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101 there have been reports of hypersensitivity pneumonitis[42] and IA[43–47] in apparently

102 immunocompetent individuals following gardening activities, however, no clinical links following

103 exposure to triazole resistant spores have been documented.

104 The aims of this study were to a) determine the numbers of triazole-susceptible and resistant *A.*

105 *fumigatus* spores in soil samples collected from residential gardens in the UK, b) characterise the

106 *cyp51A* polymorphisms responsible for resistance, and c) find environmental variables associated

107 with presence/numbers of *A. fumigatus* spores in soil samples. In order to simultaneously sample a

108 wide range of UK gardens, we were assisted by a network of citizen-scientists trained in the

109 collection of samples that may contain *A. fumigatus*. Our aim was to ascertain whether gardening

110 activities may lead to exposure to triazole-resistant genotypes of this mould that could present a risk

111 to susceptible individuals. Based on our findings, we present thoughts on how these exposure risks

112 in susceptible individuals might be mitigated.

113 **Methods**

114 *Culturing* Aspergillus fumigatus *from residential garden soil samples*

115 The soil samples from which *A fumigatus* isolates were cultured for this study were collected as part

116 of a citizen science project undertaken in June 2019, which involved 246 volunteers in the UK

117 collected a total of 509 soil samples from different locations in their gardens[48]. Participants indicated

118 on a questionnaire whether samples were collected from a border, pot or planter, compost heap,

119 bag of manure or bag of compost. Upon receipt, 2 g of each soil sample was suspended in 8 ml of

120 buffer (0.85% NaCl and 0.01% Tween 20 in distilled water), shaken vigorously and left to settle for 30

121 minutes. No adjustment was made for the moisture content of the soil when weighing it out. One

122 aliquot of 200 µl from the surface of the buffer was spread onto a plate containing sabouraud

123 dextrose agar (SDA), penicillin (200 mg/L) and streptomycin (400 mg/L) and a second aliquot of 200

124 µl was spread onto a plate containing SDA, penicillin (200 mg/L), streptomycin (400 mg/L) and

125 tebuconazole (6 mg/L). The concentration of 6 mg/L tebuconazole was chosen after testing the

126 growth of 30 isolates with known *CYP51A* mutations on SDA supplemented with 0 mg/L, 4 mg/L, 6

127 mg/L, 8 mg/L and 16 mg/L tebuconazole. The only concentration that showed no growth of any

128 isolates without *CYP51A* mutations and partial or full growth of all isolates with *CYP51A* mutations

129 was 6 mg/L. Both plates were incubated at 37°C for 48 hours, the number of colonies that

130 morphologically resembled *A. fumigatus* on each plate recorded, and the colonies growing on the

131 plate containing tebuconazole were picked into tubes containing mould preservation solution (0.2%

132 agar and 0.05% Tween 20 in deionized water) and stored at 4°C. These isolates were subsequently

133 cryopreserved in 50% glycerol solution and were DNA extracted as detailed in Boyle *et al.* (2004)[49].

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134 *Sequencing of* A. fumigatus cyp51A *gene*

135 The promoter region of *cyp51A* was amplified using forward primer 5'-
136 GGACTGGCTGATCAAACTATGC-3' and reverse primer 5'-GTTCTGTTCGGTTCCAAAGCC-3' and the
137 following PCR conditions: 95°C for five minutes; 30 cycles of 98°C for 20 seconds, 65°C for 30
138 seconds and 72°C for 30 seconds; followed by 72°C for five minutes. The PCR reaction volume used
139 was 50 µl: 10 µl of FIREPol® DNA polymerase (Solis Biodyne, Estonia), 10 µl of forward primer (1.5
140 µM; Invitrogen, US), 10 µl of reverse primer (1.5 µM; Invitrogen, US), 18 µl of nuclease-free water
141 (Merck, Germany) and 2 µl of DNA. Amplicons were visualised by gel electrophoresis and samples
142 with visible bands were sent for sequencing using the forward primer. The coding region of *cyp51A*
143 was amplified using forward primer 5'-ATGGTGCCGATGCTATGG-3' and reverse primer 5'-
144 CTGTCTCACTTGGATGTG-3' and the following PCR conditions: 94°C for two minutes; 35 cycles of 94°C
145 for 30 seconds, 60°C for 45 seconds and 72°C for 45 seconds; followed by 72°C for five minutes. The
146 PCR reaction volume used was 50 µl: 0.2 µl of Q5® high-fidelity DNA polymerase (New England
147 Biolabs, UK), 10 µl of Q5® reaction buffer (5X; New England Biolabs, UK), 0.5 µl of deoxynucleotide
148 (dNTP) solution mix (40 µM; New England Biolabs, UK), 1 µl of forward primer (10 µM; Invitrogen,
149 US), 1 µl of reverse primer (10 µM; Invitrogen, US), 35.3 µl of nuclease-free water (Merck, Germany)
150 and 2 µl of DNA. Amplicons were visualised by gel electrophoresis and samples with visible bands
151 were sent for sequencing using the Sanger chain termination method in two segments using the
152 primers 5'-TACGTTGACATCATCAATCAG-3' and 5'-GATTCACCGAACTTTCAAGGCTCG-3'. Sequences
153 were aligned using Molecular Evolutionary Genetics Analysis (MEGA) software (Penn State
154 University, US).

155 *Identification of isolates*

156 For isolates that failed to sequence using the primers for the promoter and coding regions of
157 *cyp51A,* part of the beta-tubulin gene was sequenced using forward primer 5'-
158 AATTGGTGCCGCTTTCTGG-3' and reverse primer 5'-AGTTGTCGGGACGGAATAG-3' and the following
159 PCR conditions: 94°C for 3 minutes; 30 cycles of 94°C for 15 seconds, 55°C for 30 seconds, 68°C for
160 30 seconds; followed by 68°C for 3 minutes. Amplicons were visualised by gel electrophoresis and
161 samples with visible bands were sent for sequencing using the forward primer. Basic Local Alignment
162 Search Tool (BLAST) was used to align the sequences to those in the National Center for
163 Biotechnology Information (NCBI; Bethesda, US) to identify the isolate.

164 *Environmental variables that may influence growth of* Aspergillus fumigatus

165   Table 1 details the environmental variables that were ascertained for the locations in the UK from

166   which soil samples were collected, on the date when sampling occurred, and the source from which

167   the data were obtained.

168   *Generalised linear models*

169   Generalised linear models (GLMs) were run using R version 4.0.0 to find associations between the

170   environmental variables in Table 1 and 1) the likelihoods of a sample growing susceptible or triazole-

171   resistant *A. fumigatus*, and 2) the number of susceptible or triazole-resistant *A. fumigatus* colonies

172   grown from a sample. Growth of susceptible or triazole-resistant *A. fumigatus* from a sample was

173   categorised as 0/1 and logistic regressions ("glm" function; family = "binomial") were performed.

174   The numbers of susceptible and triazole-resistant *A. fumigatus* colonies grown from samples were

175   over-dispersed; therefore negative binomial regressions (library "MASS"; "glm.nb" function) were

176   performed. Environmental variables were included in the regression model based on a significant

177   improvement on the null model, as determined by analysis of variance (ANOVA) using chi-squared

178   test. Results were considered significant when p <= 0.05. The regression model with the best fit was

179   chosen based on a reduced Akaike information criterion (AIC) score and a significant improvement

180   on the null model.

181   **Results**

182   *Susceptible and tebuconazole-resistant* A. fumigatus *in soil samples*

183   Of the 509 soil samples collected, 327 (64%) samples between them grew 5,174 *A. fumigatus*

184   isolates and 101 (20%) samples grew 736 tebuconazole-resistant isolates (Table 2). The majority of

185   samples (n = 451; 89%) were assigned a single location in the garden from which they were

186   collected, whereas the remainder were assigned multiple locations. These multiple locations

187   occurred when a border or pot/planter had recently been topped up with manure or compost. The

188   concentration of spores and mycelial fragments averaged across the samples that grew *A. fumigatus*

189   was 316 CFU/g, which ranged from 0 CFU/g in the sample collected from a border plus manure bag

190   to 600 CFU/g in the sample collected from a manure bag. The concentration of spores and mycelial

191   fragments averaged across the samples that grew tebuconazole-resistant *A. fumigatus* was 146

192   CFU/g, which ranged from 0 CFU/g in samples collected from several garden locations to 214 CFU/g

193   in samples collected from compost heaps. Figure 1 shows the geographical locations in the UK that

194   soil samples were collected from.

195   Cyp51A *polymorphisms in tebuconazole-resistant* A. fumigatus *isolates*

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196 Of the 736 tebuconazole-resistant *A. fumigatus* isolates, 93 (13%) failed to re-grow from refrigerated

197 storage for cryopreservation and DNA extraction. In the 643 isolates that re-grew, TR_{34}/L98H was

198 detected in 542 (85%), TR_{46}/Y121F/T289A in 16 (3%), TR_{53} in two,

199 $(TR_{48})^2$/Y121F/M172I/T289A/G448S in one and no *cyp51A* polymorphisms were detected in 27 (4%)

200 isolates. 14 isolates failed to sequence with the *cyp51A* promoter and coding region primers and

201 beta-tubulin sequencing confirmed their identities as *A. fischeri* (n = 8), *A. fumigatus* (n = 2), *A.*

202 *oerlinghausensis* (n = 3) and unknown (n = 1). Uncommon polymorphisms detected were TR_{34}

203 without accompanying amino acid substitutions in three isolates, $(TR_{34})^2$/L98H in one isolate and

204 $(TR_{130})^3$/D430G in four isolates. The remaining isolates contained one or more amino acid

205 substitutions in cyp51A, with or without accompanying TRs (Table 3). Further details of the

206 tebuconazole-resistant *A. fumigatus* isolates can be found in Supplementary Table 1.

207 *Environmental variables influencing growth and numbers of* A. fumigatus *colonies*

208 *Growth of* A. fumigatus *from soil samples*

209 Eight samples were excluded from the logistic regression with growth of *A. fumigatus* as the

210 outcome, which left 501 samples in the analysis. These samples were excluded because the SDA

211 plates were too contaminated to determine presence of *A. fumigatus.* Location in the garden from

212 which the soil sample was collected was the only variable that significantly affected whether a

213 sample grew *A. fumigatus* (x^2 = 67.3, df = 12, p < 0.01). The odds ratios and p-values from the logistic

214 regression model are shown in Table 4. Samples collected from a compost bag, compost heap,

215 pot/planter and pot/planter plus compost bag had significantly increased odds of growing *A.*

216 *fumigatus* (p < 0.01) compared to samples collected from a border. There were no significant

217 changes in odds of growing *A. fumigatus* from other sampling locations.

218 *Number of* A. fumigatus *colonies* grown *from soil samples*

219 The first negative binomial regression was run on the 335 samples that grew *A. fumigatus*. The only

220 variable found to significantly affect the number of *A. fumigatus* colonies grown from a sample was

221 garden location from which the sample was collected (x^2 = 50.8, df = 11, p < 0.01). In the regression

222 model, samples collected from compost bag (p < 0.01), compost heap (p < 0.01) and pot/planter plus

223 compost bag (p = 0.02) grew significantly more *A. fumigatus* colonies than samples collected from

224 borders. Samples collected from a pot/planter plus compost bag plus manure bag grew fewer *A.*

225 *fumigatus* colonies than samples collected from borders, although this reduction was marginally

226 significant (p = 0.05).

227 *Growth of tebuconazole-resistant* A. fumigatus *from soil samples*

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228   All 509 soil samples were included in the logistic regression with growth of tebuconazole-resistant *A.*

229   *fumigatus* as the outcome. The only variable found to significantly affect whether a sample grew

230   tebuconazole-resistant *A. fumigatus* was garden location from which the sample was collected (χ^2 =

231   43.0, df = 12, *p* < 0.01). The odds ratios and p-values from the logistic regression model are shown in

232   Table 5. Samples collected from a compost bag, compost heap, pot/planter and pot/planter plus

233   compost bag had significantly increased odds of growing tebuconazole-resistant *A. fumigatus* (*p* <

234   0.01) compared to samples collected from a border. There were no significant changes in odds of

235   growing tebuconazole-resistant *A. fumigatus* from other sampling locations.

236   *Number of tebuconazole-resistant* A. fumigatus *colonies* grown *from soil samples*

237   The second negative binomial regression was run on the 101 samples that grew tebuconazole-

238   resistant *A. fumigatus*. None of the environmental variables were found to have a significant effect

239   on the outcome.

Discussion

241   In this study, 5,174 *A. fumigatus* isolates were cultured from 509 soil samples collected by 249

242   citizen scientists from their gardens across the UK[48]. Of these soil samples, 327 (64%) grew *A.*

243   *fumigatus* isolates and 101 (20%) grew isolates that were resistant to tebuconazole at a

244   concentration of 6 mg/L. The percentage of soils that grew *A. fumigatus* in this study was lower than

245   the 78% of soils collected by Sewell *et al.* (2019) from several sites across South West England,

246   including parks, cemeteries, public gardens, flower beds outside hospitals, a lavender farm, a forest

247   and farmland[50]. However, the percentage of soils in this study that grew tebuconazole-resistant *A.*

248   *fumigatus* isolates was greater than the 6% of soils in Sewell *et al.* (2019) that grew *A. fumigatus*

249   with increased minimum inhibitory concentrations (MICs) to ITZ, VCZ and/or PCZ[50]. Of the 5,174 *A.*

250   *fumigatus* isolates cultured in this study, 736 (14%) were resistant to tebuconazole, which is greater

251   than the 6% prevalence of triazole-resistant *A. fumigatus* reported by Tsitsopoulou *et al.* (2018) from

252   urban and rural soils in South Wales[51] and the absence of triazole-resistance detected by van der

253   Torre *et al.* (2020) in isolates cultured from soils adhered to vegetables grown in the UK[52]. This

254   prevalence of 14% is also greater than the 9% in experimental cropland and 12% in commercial

255   wheat fields in the UK reported by Fraaije *et al.* (2020)[53]; however it is less than the 37% prevalence

256   in isolates cultured from flower bulbs bought from a garden centre in Dublin reported by Dunne *et*

257   *al.* (2017)[54]. In this study, the average concentration of *A. fumigatus* from positive soil samples was

258   316 CFU/g, which is higher than the 43.5 CFU/g in agricultural soils and 106 CFU/g in urban soils

259   from Greater Manchester reported by Bromley *et al.* (2014)[55] and considerably higher than the 0-10

260   CFU/g reported from woodlands, grass verges, experimental cropland and commercial wheat fields

261 across the UK by Fraaije *et al.* (2020)[53]. Given that *A. fumigatus* is often considered to be ubiquitous

262 in the environment, it is intriguing that 36% of the soil samples collected in this study did not grow

263 this mould. We speculate that *A. fumigatus* spores and mycelial fragments in garden soils are killed

264 by triazole residues from dipped bulbs[56], for example, if they have not developed triazole-resistance.

265 It is also possible that *A. fumigatus* is out-competed by other microbes, especially in soils that have

266 not experienced the high temperatures that are associated with composting.

267 Of the 736 *A. fumigatus* isolates that grew on tebuconazole at 6 mg/L, 93 (13%) did not re-grow

268 from short-term storage in the fridge, which left 643 (87%) isolates for sequencing of the *cyp51A*

269 promoter and gene coding regions. Similar to existing UK studies[50,51,55], the predominant mutation

270 identified in this study was TR_{34}/L98H (n = 535; 73%). Of these isolates, 22 had amino acid

271 substitutions in cyp51A in addition to L98H. Six isolates had T289A, I364V and G448S amino acid

272 substitutions in addition to TR_{34}/L98H; which has been previously detected in Korea in a patient with

273 IA[57] and in Japan on tulip bulbs imported from The Netherlands[58]. TR_{68}/L98H was detected in one

274 isolate, which was found to be two repeats of the 34-base pair (bp) insert, and in three isolates TR_{34}

275 was detected without any accompanying amino acid substitutions, which was first detected in an

276 environmental isolate collected from Scotland[59]. TR_{46}/Y121F/T289A was detected in 16 (2%) isolates

277 and was accompanied by S363P, I364V and G448S in four additional isolates; a combination reported

278 from the Netherlands in 2018[53]. Additional polymorphisms detected in this study included TR_{53},

279 which has been previously reported from flower fields in Colombia[60] and from a patient with

280 multiple-azole-resistant *A. fumigatus* osteomyelitis in The Netherlands[61], and

281 TR_{92}/Y121F/M172I/T289A/G448S, which has been previously detected in flower bulb waste in The

282 Netherlands[38] and is two repeats of the 46 bp insert. There were 33 (4%) isolates in this study that

283 did not contain any TRs: five contained I242V, one contained C270R and 27 had no amino acid

284 substitutions in cyp51A. I242V is the only single cyp51A amino acid substitution detected in this

285 study to have been reported in studies summarising *cyp51A* polymorphisms[62–65], which may suggest

286 these polymorphisms occurred *in situ*. The 28 isolates that did not contain any *cyp51A*

287 polymorphisms may well be using non-*cyp51A* mechanisms for triazole-resistance, such as

288 overexpression of efflux pumps, *cyp51B* overexpression, cholesterol import or *HapE* mutation, which

289 were not explored in this study[66].

290 The only environmental variable measured in this study that was found to have a significant effect

291 on whether a sample grew *A. fumigatus*, or on the numbers of *A. fumigatus* grown, was the garden

292 location from which the sample was collected. The greatest concentration of *A. fumigatus* was

293 cultured from a bag of manure at 600 CFU/g, followed by homemade compost heap samples at 505

294 CFU/g, commercial compost bag samples at 451 CFU/g and pot/planters containing commercial

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295   compost at 321 CFU/g. Soil samples that did not contain compost grew fewer *A. fumigatus* isolates:

296   254 CFU/g from pot/planters and 204 CFU/g from borders. Similar observations were made for

297   tebuconazole-resistant *A. fumigatus*, with concentrations of 128-289 CFU/g recorded for samples

298   containing compost and 98-127 CFU/g for samples without compost. As citizen scientists were only

299   asked to indicate one garden location from which the soil sample was collected, it is possible that

300   the concentrations of *A. fumigatus* spores from borders and pot/planters were inflated by the recent

301   addition of compost that was not indicated on the questionnaire. In the regression models, soils

302   collected from commercial compost bags, homemade compost heaps, pot/planters and pot/planters

303   plus commercial compost had significantly greater odds of growing *A. fumigatus* and tebuconazole-

304   resistant *A. fumigatus* (p < 0.01 for all associations) when compared to soil samples collected from

305   borders. Furthermore, samples collected from commercial compost bags, homemade compost

306   heaps and pot/planter plus commercial compost grew significantly more *A. fumigatus* colonies

307   compared to samples collected from borders. No association was found for garden location sampled

308   from and numbers of tebuconazole-resistant *A. fumigatus*.

309   Several existing studies have looked for triazole-resistant *A. fumigatus* specifically in compost in the

310   UK and globally, and the findings have been highly variable. Tsitsopoulou *et al.* (2018) collected 11

311   compost samples from agricultural fields, a horticultural nursery and public areas across South

312   Wales that grew 10 *A. fumigatus* isolates in total; none of which were triazole-resistant[51]. Dunne *et*

313   *al.* (2017) do not report how many samples they collected from commercial compost bought from a

314   garden centre in Dublin or how many *A. fumigatus* were cultured from these samples; only that one

315   isolate was triazole-resistant[56]. Sewell *et al.* (2019) collected two samples from a compost heap in

316   London that, combined with three samples collected from a flower bed ~500m away, gave a 60%

317   prevalence of triazole-resistant *A. fumigatus*[50]. Pugliese *et al.* (2018) sampled from composting

318   orange peel in Italy and found *A. fumigatus* concentrations of 8.8 x 10^3 CFU/g at the start of the

319   process rising to 605.7 x 10^3 CFU/g by the end, yet none of the 30 isolates selected for susceptibility

320   testing were triazole-resistant[67]. Santoro *et al.* (2017) sampled from 11 green and brown composts

321   across Spain, Hungary and Italy and found concentrations of *A. fumigatus* ranging from 100 to 10.6 x

322   10^3 CFU/g, yet none of the 30 isolates selected for susceptibility testing were triazole-resistant[68].

323   Ahangarkani *et al.* (2020) screened isolates cultured from 300 compost samples collected in Iran and

324   detected 57 isolates with elevated MICs to ITZ and VCZ[69]. Zhang *et al.* (2021) collected 114 samples

325   from a plant waste stockpile over 16 months in the Netherlands and detected >10^3 *A. fumigatus*

326   CFU/g in 74% of samples, with the prevalence of triazole-resistant *A. fumigatus* averaging 50%

327   across all samples[37]. Also in the Netherlands, Schoustra *et al.* (2019) found concentrations of

328   triazole-resistant *A. fumigatus* of 200 CFU/g in household green waste, 1.5-1.8 x 10^3 CFU/g in

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329   compost heaps in residential gardens, up to 2.3 x 10^5 CFU/g in flower bulb waste and up to 8.4 x 10^4

330   CFU/g in organic waste from landscaping[38].

331   The key findings of this study are that 64% of soil samples collected from residential gardens in the

332   UK grew *A. fumigatus* and 20% of samples grew tebuconazole-resistant *A. fumigatus*. This means

333   that individuals are very likely to be exposed to both *A. fumigatus* and triazole-resistant *A. fumigatus*

334   spores that are aerosolised from soil when they are undertaking gardening activities[43–47]. Although

335   this study has not undertaken susceptibility testing for the tebuconazole-resistant *A. fumigatus*

336   isolates against medical triazoles, the most commonly detected *cyp51A* polymorphisms TR_{34}/L98H

337   and TR_{46}/Y121F/T289A are associated with elevated MICs to ITZ, PCZ and VCZ[70]. Furthermore,

338   Hodiamont *et al.* (2009) reported a clinical isolate containing TR_{53} as being resistant to ITR and VCZ,

339   with reduced susceptibility to PCZ[61]. This study also reports that the likelihood of being exposed to *A.*

340   *fumigatus* and triazole-resistant *A. fumigatus* spores is significantly greater when handling

341   commercial or homemade compost compared to soils in borders or pots/planters. The 14%

342   prevalence of triazole-resistance detected in garden soil samples in this study is higher than most

343   existing studies that have sampled from rural and urban locations in the UK, which is likely being

344   driven by the concentrated application of compost in residential settings. The National Aspergillosis

345   Centre advises that people take care when opening bags of compost and recommends wearing a

346   facemask whilst doing so to avoid dust inhalation. Currently, the only health warning on commercial

347   compost bags is for women to not handle compost without gloves if they are pregnant, presumably

348   to avoid toxoplasmosis infection[71]. The evidence presented here supports the recommendation for

349   users to wear a mask whilst handling compost and the introduction of health warnings on bags of

350   compost with regards to inhaling *A. fumigatus*. Measures could also be taken by compost producers

351   to sterilise the composting before packaging, thereby killing viable *A. fumigatus* spores and

352   eliminating the immediate hazard it poses to the user.

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Competing Interests

363 The authors have no competing interests to declare.

Author Contributions

365 JMGS, ACS, and MCF conceptualised the study; AA and PSD contributed experimental techniques;

366 JMGS and RC processed samples; JMGS and CU analysed the data; JMGS drafted the original

367 manuscript, which CU, ACS and MCF reviewed and edited.

References

368

369   1.   Millner, P. D., Marsh, P. B., Snowden, R. B. & Parr, J. F. Occurrence of Aspergillus fumigatus
370   during composting of sewage sludge. *Appl. Environ. Microbiol.* **34**, 765–772 (1977).

371   2.   Beffa, T., Staib, F., Lott Fischer, J., Lyon, P. F., Gumowski, P., Marfenina, O. E., Dunoyer-
372   Geindre, S., Georgen, F., Roch-Susuki, R., Gallaz, L. & Latgé, J. P. Mycological control and
373   surveillance of biological waste and compost. *Med. Mycol.* **36**, 137–45 (1998).

374   3.   Kwon-Chung, K. J. & Sugui, J. A. Aspergillus fumigatus- What Makes the Species a Ubiquitous
375   Human Fungal Pathogen? *PLoS Pathog.* **9**, 1–4 (2013).

376   4.   Latgé, J. P. Aspergillus fumigatus and Aspergillosis. *Clin. Microbiol. Rev.* **12**, 310–350 (1999).

377   5.   Moss, R. B. Treatment options in severe fungal asthma and allergic bronchopulmonary
378   aspergillosis. *Eur. Respir. J.* **43**, 1487–1500 (2014).

379   6.   Hohl, T. M. & Feldmesser, M. Aspergillus fumigatus: Principles of pathogenesis and host
380   defense. *Eukaryot. Cell* **6**, 1953–1963 (2007).

381   7.   Arias, M., Santiago, L., Vidal-García, M., Redrado, S., Lanuza, P., Comas, L., Domingo, M. P.,
382   Rezusta, A. & Gálvez, E. M. Preparations for invasion: Modulation of host lung immunity
383   during pulmonary aspergillosis by gliotoxin and other fungal secondary metabolites. *Front.*
384   *Immunol.* **9**, 1–12 (2018).

385   8.   Maghrabi, F. & Denning, D. W. The Management of Chronic Pulmonary Aspergillosis: The UK
386   National Aspergillosis Centre Approach. *Curr. Fungal Infect. Rep.* **11**, 242–251 (2017).

387   9.   Lin, S., Schranz, J. & Teutsch, S. M. Aspergillosis Case-Fatality Rate : Systematic Review of the
388   Literature. **60612**, (2001).

389   10.   Schauwvlieghe, A. F. A. D., Rijnders, B. J. A., Philips, N., Verwijs, R., Vanderbeke, L., Tienen, C.
390   Van, Lagrou, P. K., Verweij, P. P. E., Veerdonk, F. L. Van De, Gommers, P. D., Spronk, P.,
391   Bergmans, D. C. J. J., Hoedemaekers, A., Andrinopoulou, E., Berg, C. H. S. B. Van Den,
392   Juffermans, P. N. P., Hodiamont, C. J., Vonk, A. G., Depuydt, P. P., *et al.* Invasive aspergillosis
393   in patients admitted to the intensive care unit with severe influenza : a retrospective cohort
394   study. *Lancet Respir.* **6**, 782–792 (2018).

395   11.   Armstrong-James, D., Youngs, J., Bicanic, T., Abdolrasouli, A., Denning, D. W., Johnson, E.,
396   Mehra, V., Pagliuca, T., Patel, B., Rhodes, J., Schelenz, S., Shah, A., Van de Veerdonk, F. L.,
397   Verweij, P. E., Lewis White, P. & Fisher, M. C. Confronting and mitigating the risk of COVID-19

13

398   associated pulmonary aspergillosis. *Eur. Respir. J.* **56**, (2020).

399   12.   Pegorie, M., Denning, D. W. & Welfare, W. Estimating the burden of invasive and serious

400   fungal disease in the United Kingdom. *J. Infect.* **74**, 60–71 (2017).

401   13.   Bueid, A., Howard, S. J., Moore, C. B., Richardson, M. D., Harrison, E., Bowyer, P. & Denning,

402   D. W. Azole antifungal resistance in Aspergillus fumigatus: 2008 and 2009. *J. Antimicrob.*

403   *Chemother.* **65**, 2116–2118 (2010).

404   14.   van der Linden, J. W. M., Snelders, E., Kampinga, G. A., Rijnders, B. J. A., Mattsson, E., Debets-

405   Ossenkopp, Y. J., Kuijper, E. J., van Tiel, F. H., Melchers, W. J. G. & Verweij, P. E. Clinical

406   implications of azole resistance in Aspergillus fumigatus, the Netherlands, 2007-2009. *Emerg.*

407   *Infect. Dis.* **17**, 1846–1854 (2011).

408   15.   Meis, J. F., Chowdhary, A., Rhodes, J. L., Fisher, M. C. & Verweij, P. E. Clinical implications of

409   globally emerging azole resistance in Aspergillus fumigatus. *Philos. Trans. B* **371**, 1–10 (2016).

410   16.   Snelders, E., Camps, S. M. T., Karawajczyk, A., Schaftenaar, G., Kema, G. H. J., van der Lee, H.

411   A., Klaassen, C. H., Melchers, W. J. G. & Verweij, P. E. Triazole fungicides can induce cross-

412   resistance to medical triazoles in Aspergillus fumigatus. *PLoS One* **7**, (2012).

413   17.   Kleinkauf, N., Verweij, P. E., Arendrup, M. C., Donnelly, P. J., Cuenca-Estrella, M., Fraaije, B.,

414   Melchers, W. J. G., Adriaenssens, N., Kema, G. H. J., Ullmann, A., Bowyer, P. & Denning, D. W.

415   *European Centre for Disease Prevention and Control: Risk assessment on the impact of*

416   *environmental usage of triazoles on the development and spread of resistance to medical*

417   *triazoles in Aspergillus species*. (2013).

418   18.   Defra. Government Review of Waste Policy in England 2011. *Gov. Waste Policy Rev.* 1–80

419   (2011).

420   19.   HM Government. *Food 2030*. (2010).

421   20.   Sykes, P., Jones, K. & Wildsmith, J. D. Managing the potential public health risks from

422   bioaerosol liberation at commercial composting sites in the UK: An analysis of the evidence

423   base. *Resour. Conserv. Recycl.* **52**, 410–424 (2007).

424   21.   Deacon, L., Pankhurst, L., Liu, J., Drew, G. H., Hayes, E. T., Jackson, S., Longhurst, J., Longhurst,

425   P., Pollard, S. & Tyrrel, S. Endotoxin emissions from commercial composting activities.

426   *Environ. Heal. A Glob. Access Sci. Source* **8**, 2–5 (2009).

427   22.   Gilbert, E. J., Adrian, K., Karnon, J. D., Swan, J. R. & Crook, B. Preliminary Results of

14

428   Monitoring the Release of Bioaerosols from Composting Facilities in the UK: Interpretation,

429   Modelling and Appraisal of Mitigation Measures. *Biocycle* (2002).

430   23.   Sánchez-Monedero, M. A. & Stentiford, E. I. Generation and dispersion of airborne

431   microorganisms from composting facilities. *Process Saf. Environ. Prot.* **81**, 166–170 (2003).

432   24.   Stagg, S., Bowry, A., Kelsey, A. & Crook, B. *Bioaerosol emissions from waste composting and*

433   *the potential for workers ' exposure*. *Health and Safety Executive* (2010).

434   25.   Taha, M. P. M., Drew, G. H., Longhurst, P. J., Smith, R. & Pollard, S. J. T. Bioaerosol releases

435   from compost facilities: Evaluating passive and active source terms at a green waste facility

436   for improved risk assessments. *Atmos. Environ.* **40**, 1159–1169 (2006).

437   26.   Taha, M. P. M., Pollard, S. J. T., Sarkar, U. & Longhurst, P. Estimating fugitive bioaerosol

438   releases from static compost windrows: Feasibility of a portable wind tunnel approach.

439   *Waste Manag.* **25**, 445–450 (2005).

440   27.   Wery, N. Bioaerosols from composting facilities - a review. *Front. Cell. Infect. Microbiol.* **4**, 1–9

441   (2014).

442   28.   Schantora, A. L., Casjens, S., Deckert, A., Kampen, V. van, Neumann, H.-D., Brüning, T., Raulf,

443   M., Bünger, J. & Hoffmeyer, F. *Prevalence of Work-Related Rhino-Conjunctivitis and*

444   *Respiratory Symptoms Among Domestic Waste Collectors*. *Environment Exposure to*

445   *Pollutants* (2014).

446   29.   Velasco Garrido, M., Bittner, C., Harth, V. & Preisser, A. M. Health status and health-related

447   quality of life of municipal waste collection workers - A cross-sectional survey. *J. Occup. Med.*

448   *Toxicol.* **10**, 1–7 (2015).

449   30.   Hoffmeyer, F., van Kampen, V., Taeger, D., Deckert, A., Rosenkranz, N., Kaßen, M., Schantora,

450   A. L., Brüning, T., Raulf, M. & Bünger, J. Prevalence of and relationship between

451   rhinoconjunctivitis and lower airway diseases in compost workers with current or former

452   exposure to organic dust. *Ann. Agric. Environ. Med.* **21**, 705–711 (2014).

453   31.   Van Kampen, V., Deckert, A., Hoffmeyer, F., Taeger, D., Brinkmann, E., Brüning, T., Raulf-

454   Heimsoth, M. & Bünger, J. Symptoms, spirometry, and serum antibody concentrations among

455   compost workers exposed to organic dust. *J. Toxicol. Environ. Heal. - Part A Curr. Issues* **75**,

456   492–500 (2012).

457   32.   Hambach, R., Droste, J., François, G., Weyler, J., Van Soom, U., De Schryver, A., Vanoeteren, J.

458   & van Sprundel, M. Work-related health symptoms among compost facility workers: a cross-

15

459   sectional study. *Arch. Public Heal.* **70**, 2–6 (2012).

460   33.   Athanasiou, M., Makrynos, G. & Dounias, G. Respiratory health of municipal solid waste
461   workers. *Occup. Med. (Chic. Ill).* **60**, 618–623 (2010).

462   34.   Allmers, H., Huber, H. & Baur, X. Two year follow-up of a garbage collector with allergic
463   bronchopulmonary aspergillosis (ABPA). *Am. J. Ind. Med.* **37**, 438–442 (2000).

464   35.   Poole, C. J. M. & Wong, M. Allergic bronchopulmonary aspergillosis in garden waste
465   (compost) collectors-occupational implications. *Occup. Med. (Chic. Ill).* **63**, 517–519 (2013).

466   36.   Bünger, J., Schappler-Scheele, B., Hilgers, R. & Hallier, E. A 5-year follow-up study on
467   respiratory disorders and lung function in workers exposed to organic dust from composting
468   plants. *Int. Arch. Occup. Environ. Health* **80**, 306–312 (2007).

469   37.   Zhang, J., Jimenez, L. L., Snelders, E., Debets, A. J. M., Rietveld, A. G., Verweij, P. E., Schoustra,
470   S. E. & Zwaan, B. J. Dynamics of Aspergillus fumigatus in Azole Fungicide-Containing Plant
471   Waste in the Netherlands (2016-2017). *Appl. Environ. Microbiol.* **87**, 1–12 (2021).

472   38.   Schoustra, S. E., Debets, A. J. M., Rijs, A. J. M. M., Zhang, J., Snelders, E., Leendertse, P. C.,
473   Melchers, W. J. G., Rietveld, A. G., Zwaan, B. J. & Verweij, P. E. Environmental hotspots for
474   azole resistance selection of Aspergillus fumigatus, the Netherlands. *Emerg. Infect. Dis.* **25**,
475   (2019).

476   39.   Oxford Economics. *The Economic Impact of Ornamental Horticulture and Landscaping in the*
477   *UK - A Report for the Ornamental Horticulture Round Table Group*.
478   https://www.rhs.org.uk/science/pdf/The-economic-impact-of-ornamental-horticulture-
479   and.pdf (2018).

480   40.   Déportes, I., Benoit-Guyod, J. L. & Zmirou, D. Hazard to man and the environment posed by
481   the use of urban waste compost: a review. *Sci. Total Environ.* **172**, 197–222 (1995).

482   41.   Defra. *Household Waste Prevention Evidence Review- A report for Defra's Waste and*
483   *Resources Evidence Programme*. (2009).

484   42.   Brown, J. E., Masood, D., Couser, J. I. & Patterson, R. Hypersensitivity pneumonitis from
485   residential composting: residential composter's lung. *Ann. allergy, asthma Immunol.* **74**, 45–7
486   (1995).

487   43.   Cavling Arendrup, M., Ronan O'Driscoll, B., Petersen, E. & Denning, D. W. Acute pulmonary
488   aspergillosis in immunocompetent subjects after exposure to bark chippings. *Scand. J. Infect.*

16

489   *Dis.* **38**, 945–949 (2006).

490   44.   Batard, E., Renaudin, K., Morin, O., Desjars, P. & Germaud, P. Fatal acute granulomatous
491   pulmonary aspergillosis in a healthy subject after inhalation of vegetal dust. *Eur. J. Clin.*
492   *Microbiol. Infect. Dis.* **22**, 357–359 (2003).

493   45.   Jung, N., Mronga, S., Schroth, S., Vassiliou, T., Sommer, F., Walthers, E., Aepinus, C.,
494   Jerrentrup, A., Vogelmeier, C., Holland, A. & Koczulla, R. Gardening can induce pulmonary
495   failure: Aspergillus ARDS in an immunocompetent patient, a case report. *BMC Infect. Dis.* **14**,
496   1–3 (2014).

497   46.   Russell, K., Broadbridge, C., Murray, S., Waghorn, D. & Mahoney, A. Gardening can seriously
498   damage your health. *Lancet* **371**, 2056 (2008).

499   47.   Zuk, L. A., King, D., Zakhour, H. D. & Delaney, J. C. Locally invasive pulmonary aspergillosis
500   occurring in a gardener: an occupational hazard? *Thorax* **44**, 678–679 (1989).

501   48.   Shelton, J. M. G., Fisher, M. C. & Singer, A. C. Campaign-Based Citizen Science for
502   Environmental Mycology: The Science Solstice and Summer Soil-Stice Projects to Assess Drug
503   Resistance in Air- and Soil-Borne *Aspergillus fumigatus*. *Citiz. Sci. Theory Pract.* **5**, 20 (2020).

504   49.   Boyle, D. G., Boyle, D. B., Olsen, V., Morgan, J. A. T. & Hyatt, A. D. Rapid quantitative
505   detection of chytridiomycosis (Batrachochytrium dendrobatidis) in amphibian samples using
506   real-time Taqman PCR assay. *Dis. Aquat. Organ.* **60**, 141–148 (2004).

507   50.   Sewell, T. R., Zhang, Y., Brackin, A. P., Shelton, J. M. G., Rhodes, J. & Fisher, M. C. Elevated
508   Prevalence of Azole-Resistant Aspergillus fumigatus in Urban versus Rural Environments in
509   the United Kingdom. *Antimicrob. Agents Chemother.* **63**, 1–8 (2019).

510   51.   Tsitsopoulou, A., Posso, R., Vale, L., Bebb, S., Johnson, E. & White, P. L. Determination of the
511   prevalence of triazole resistance in environmental Aspergillus fumigatus strains isolated in
512   South Wales, UK. *Front. Microbiol.* **9**, 1–8 (2018).

513   52.   van der Torre, M. H., Whitby, C., Eades, C. P., Moore, C. B., Novak-Frazer, L., Richardson, M.
514   D. & Rautemaa-Richardson, R. Absence of azole antifungal resistance in Aspergillus fumigatus
515   isolated from root vegetables harvested from UK arable and horticultural soils. *J. Fungi* **6**, 1–
516   10 (2020).

517   53.   Fraaije, B., Atkins, S., Hanley, S., Macdonald, A. & Lucas, J. The Multi-Fungicide Resistance
518   Status of Aspergillus fumigatus Populations in Arable Soils and the Wider European
519   Environment. *Front. Microbiol.* **11**, 1–17 (2020).

520 54. Dunne, K., Hagen, F., Pomeroy, N., Meis, J. F. & Rogers, T. R. Intercountry Transfer of Triazole-
521   Resistant Aspergillus fumigatus on Plant Bulbs. *Clin. Infect. Dis.* **65**, 147–149 (2017).

522 55. Bromley, M. J., Van Muijlwijk, G., Fraczek, M. G., Robson, G., Verweij, P. E., Denning, D. W. &
523   Bowyer, P. Occurrence of azole-resistant species of Aspergillus in the UK environment. *J.*
524   *Glob. Antimicrob. Resist.* **2**, 276–279 (2014).

525 56. Dunne, K., Hagen, F., Pomeroy, N., Meis, J. F. & Rogers, T. R. Intercountry Transfer of Triazole-
526   Resistant Aspergillus fumigatus on Plant Bulbs. *Clin. Infect. Dis.* **65**, (2017).

527 57. Cho, S. Y., Lee, D. G., Kim, W. B., Chun, H. S., Park, C., Myong, J. P., Park, Y. J., Choi, J. K., Lee,
528   H. J., Kim, S. H., Park, S. H., Choi, S. M., Choi, J. H. & Yoo, J. H. Epidemiology and antifungal
529   susceptibility profile of Aspergillus species: Comparison between environmental and clinical
530   isolates from patients with hematologic malignancies. *J. Clin. Microbiol.* **57**, 1–13 (2019).

531 58. Hagiwara, D. Isolation of azole-resistant Aspergillus fumigatus from imported plant bulbs in
532   Japan and the effect of fungicide treatment. *J. Pestic. Sci.* **45**, 147–150 (2020).

533 59. Rhodes, J., Abdolrasouli, A., Dunne, K., Sewell, T. R., Zhang, Y., Ballard, E., Brackin, A. P., Rhijn,
534   N. van, Tsitsopoulou, A., Posso, R. B., Chotirmall, S. H., McElvaney, N. G., Murphy, P. G.,
535   Talento, A. F., Renwick, J., Dyer, P. S., Szekely, A., Bromley, M. J., Johnson, E. M., *et al.* Tracing
536   patterns of evolution and acquisition of drug resistant Aspergillus fumigatus infection from
537   the environment using population genomics. *bioRxiv* 1–57 (2021).

538 60. Alvarez-Moreno, C., Lavergne, R. A., Hagen, F., Morio, F., Meis, J. F. & Le Pape, P. Azole-
539   resistant Aspergillus fumigatus harboring TR34/L98H, TR46/Y121F/T289A and TR53
540   mutations related to flower fields in Colombia. *Sci. Rep.* **7**, (2017).

541 61. Hodiamont, C. J., Dolman, K. M., Ten berge, I. J. M., Melchers, W. J. G., Verweij, P. E. & Pajkrt,
542   D. Multiple-azole-resistant Aspergillus fumigatus osteomyelitis in a patient with chronic
543   granulomatous disease successfully treated with long-term oral posaconazole and surgery.
544   *Med. Mycol.* **47**, 217–220 (2009).

545 62. Rybak, J. M., Fortwendel, J. R. & Rogers, P. D. Emerging threat of triazole-resistant Aspergillus
546   fumigatus. *J. Antimicrob. Chemother.* **74**, 835–842 (2019).

547 63. Howard, S. J., Cerar, D., Anderson, M. J., Albarrag, A., Fisher, M. C., Pasqualotto, A. C.,
548   Laverdiere, M., Arendrup, M. C., Perlin, D. S. & Denning, D. W. Frequency and evolution of
549   azole resistance in Aspergillus fumigatus associated with treatment failure. *Emerg. Infect. Dis.*
550   **15**, 1068–1076 (2009).

551 64. van der Torre, M. H., Novak-Frazer, L. & Rautemaa-Richardson, R. Detecting azole-antifungal
552   resistance in Aspergillus fumigatus by pyrosequencing. *J. Fungi* **6**, 1–15 (2020).

553 65. Bernal-Martínez, L., Alastruey-Izquierdo, A. & Cuenca-Estrella, M. Diagnostics and
554   susceptibility testing in Aspergillus. *Future Microbiol.* **11**, 315–328 (2016).

555 66. Chowdhary, A., Sharma, C., Hagen, F. & Meis, J. F. Exploring azole antifungal drug resistance
556   in Aspergillus fumigatus with special reference to resistance mechanisms. *Future Microbiol.* **9**,
557   697–711 (2014).

558 67. Pugliese, M., Matić, S., Prethi, S., Gisi, U. & Gullino, M. L. Molecular characterization and
559   sensitivity to demethylation inhibitor fungicides of Aspergillus fumigatus from orange-based
560   compost. *PLoS One* **13**, 1–18 (2018).

561 68. Santoro, K., Matić, S., Gisi, U., Spadaro, D., Pugliese, M. & Gullino, M. L. Abundance, genetic
562   diversity and sensitivity to demethylation inhibitor fungicides of Aspergillus fumigatus
563   isolates from organic substrates with special emphasis on compost. *Pest Manag. Sci.* **73**,
564   2481–2494 (2017).

565 69. Ahangarkani, F., Puts, Y., Nabili, M., Khodavaisy, S., Moazeni, M., Salehi, Z., Laal Kargar, M.,
566   Badali, H. & Meis, J. F. First azole-resistant Aspergillus fumigatus isolates with the
567   environmental TR46/Y121F/T289A mutation in Iran. *Mycoses* **63**, 430–436 (2020).

568 70. Buil, J. B., Hagen, F., Chowdhary, A., Verweij, P. E. & Meis, J. F. Itraconazole, voriconazole, and
569   posaconazole CLSI MIC distributions for wild-type and azole-resistant Aspergillus fumigatus
570   isolates. *J. Fungi* **4**, 1–9 (2018).

571 71. Jones, J. L., Krueger, A., Schulkin, J. & Schantz, P. M. Toxoplasmosis prevention and testing in
572   pregnancy, survey of obstetrician-gynaecologists. *Zoonoses Public Health* **57**, 27–33 (2010).

573 72. Met Office. HadUK-Grid datasets. https://www.metoffice.gov.uk/research/climate/maps-
574   and-data/data/haduk-grid/datasets.

575 73. UKCEH: Land Cover Map 2019. https://catalogue.ceh.ac.uk/documents/31f4887a-1691-4848-
576   b07c-61cdc468ace7.

577 74. QGIS. https://www.qgis.org/en/site/.

578 75. Scottish Environment Protection Agency (SEPA): Waste Sites. https://www.sepa.org.uk/data-
579   visualisation/waste-sites-and-capacity-tool/ (2019).

580 76. Natural Resources Wales: Environmental Permitting Regulations – Waste Sites.

19

581   http://lle.gov.wales/catalogue/item/EnvironmentalPermittingRegulationsWasteSites/?lang=e

582   n (2021).

583 77. Northern Ireland Environment Agency: Waste Licenses Register. https://appsd.daera-

584   ni.gov.uk/wastelicences/.

585 78. Team, R. C. R: A language and environment for statistical computing. R Foundation for

586   Statistical Computing, Vienna, Austria. https://www.r-project.org/ (2020).

587 79. Snelders, E., Van Der Lee, H. A. L., Kuijpers, J., Rijs, A. J. M. M., Varga, J., Samson, R. A.,

588   Mellado, E., Donders, A. R. T., Melchers, W. J. G. & Verweij, P. E. Emergence of azole

589   resistance in Aspergillus fumigatus and spread of a single resistance mechanism. *PLoS Med.* **5**,

590   1629–1637 (2008).

591 80. Majima, H., Teppei, A., Watanabe, A. & Kamei, K. D430G mutation of cyp51A in Aspergillus

592   fumigatus causes azole-resistance. in *9th Advances Against Aspergillosis and Mucormycosis*

593   90 (2020).

594

595 **Figure 1:** Geographical locations in the UK that soil samples were collected from by citizen scientists.
596 Blue dots indicate samples that did not grow *Aspergillus fumigatus*, green dots indicate samples that
597 grew *Aspergillus fumigatus* and red dots indicate samples that grew tebuconazole-resistant *A.*
598 *fumigatus*. Base maps created using data obtained from OpenStreetMap (CC BY-SA 4.0); URL:
599 https://www.openstreetmap.org.

Environmental variables ascertained for sampling date and location	Source of information
Garden location that soil sample was collected from	Citizen scientist
Date that sample was collected	Citizen scientist
Maximum daily temperature at sampling location on sampling date	Met Office HadUK-Grid dataset[72]
Land cover classification of sampling location	UKCEH Land Cover Map 2019[73]
Urban or rural classification of sampling location	Calculated from land cover classification
Percentage of arable land in 2km buffer surrounding sampling location	Calculated from UKCEH Land Cover Map 2019 using QGIS 3.16.4[74]
Distance of sampling location to nearest composter with open windrow or outdoor activity	Composter locations obtained from Environment Agency, Scottish Environment Agency (SEPA) website[75], Natural Resources Wales website[76] and Northern Ireland Environment Agency website[77]. Distances calculated using package "geosphere" in R version 4.0.0[78].

Table 1: Environmental variables obtained for soil sampling locations and dates and the sources they were obtained from.

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Location in garden that soil sample was collected from	Number of soil samples	Samples that grew *A. fumigatus* (% of samples)	Samples that grew tebuconazole-resistant *A. fumigatus* (% of samples)	Number of *A. fumigatus* isolates grown	Average *A. fumigatus* CFU/g	Number of tebuconazole-resistant *A. fumigatus* isolates grown (% of *A. fumigatus* isolates)	Average tebuconazole-resistant *A. fumigatus* CFU/g
Border	206	99 (48)	19 (9)	1,009	204	121 (12)	127
+ compost bag	7	4 (57)	1 (14)	56	280	8 (14)	160
+ compost heap	5	3 (60)	0 (0)	44	293	0 (0)	0
+ manure bag	1	0 (0)	0 (0)	0	0	0 (0)	0
+ manure bag + compost heap	1	1 (100)	0 (0)	1	20	0 (0)	0
Compost bag	49	44 (90)	20 (41)	993	451	137 (14)	137
Compost heap	80	58 (73)	27 (34)	1,464	505	289 (20)	214
Manure bag	1	1 (100)	0 (0)	30	600	0 (0)	0
Pot/planter	115	79 (69)	26 (23)	1,005	254	130 (13)	98
+ compost bag	38	33 (87)	8 (21)	529	321	51 (15)	128
+ compost bag + compost heap	3	2 (67)	0 (0)	21	210	0 (0)	0
+ compost bag + manure bag	2	2 (100)	0 (0)	4	40	0 (0)	0
+ compost heap	1	1 (100)	0 (0)	18	360	0 (0)	0
Total	**509**	**327 (64)**	**101 (20)**	**5,174**	**316**	**736 (14)**	**145**

Table 2: A breakdown of the number of soil samples collected, the number and percentage of soil samples that grew susceptible and tebuconazole-resistant *Aspergillus fumigatus,* the numbers of susceptible and tebuconazole-resistant *A. fumigatus* isolates grown and the average colony forming unit per gram (CFU/g) across samples that grew susceptible and tebuconazole-resistant *A. fumigatus* by the location(s) in the garden that the soil sample was collected from.

Tandem repeat in *cyp51A* promoter region	Amino acid substitution(s) in cyp51A	Location in garden that soil sample was collected from						Total:	Medical triazole susceptibility
		B	B+CB	CB	CH	PP	PP+CB		
-	-	3			1	23		**27**	
-	C270R					1		**1**	
-	I242V					5		**5**	[65]
TR_{34}	-				1	1	1	**3**	[59]
TR_{34}	L98H	82	7	117	237	65	34	**542**	[79]
TR_{34}	L98H/Q191E			1				**1**	
TR_{34}	L98H/R196L		1					**1**	
TR_{34}	L98H/K240R	1				1		**2**	
TR_{34}	L98H/T289A/I364V/G448S				6			**6**	[57]
TR_{34}	L98H/K372R				1			**1**	
TR_{34}	L98H/P394R			1				**1**	
TR_{34}	L98H/F404C/F459S/A460S			1				**1**	
TR_{34}	L98H/F404V			1				**1**	
TR_{34}	L98H/N406D			1				**1**	
TR_{34}	L98H/N406M				1			**1**	
TR_{34}	L98H/K421R			1				**1**	
TR_{34}	L98H/P443L					2		**2**	
TR_{34}	L98H/A460S					1		**1**	
TR_{34}	L98H/D481N			1			1	**2**	
$(TR_{34})^2$	L98H	1						**1**	
TR_{46}	Y121F/M178W/T289A/S363P/I364V/G448S				1			**1**	
TR_{46}	Y121F/T289A				15		1	**16**	
TR_{46}	Y121F/T289A/S363P/I364V/G448S				4			**4**	[53]
$(TR_{46})^2$	Y121F/M172I/T289A/G448S				1			**1**	[38]
TR_{53}	-	1			1			**2**	[61]
$(TR_{130})^3$	D430G				4			**4**	[80]
Failed to sequence	Failed to sequence[a]	3			3	8		**14**	
	Total:	91	8	124	276	107	37	**643**	

Table 3: *cyp51a* polymorphisms for the 636 tebuconazole-resistant *Aspergillus fumigatus* isolates, by garden location they were collected from.

[a] Samples that failed to amplify with the *cyp51A* promoter and coding region primers were sequenced using beta-tubulin primers for fungal identification.

B=border, CB=compost bag, CH=compost heap, MB=manure bag, PP=pot/planter.

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Environmental variable	Odds ratio (95% CI)	Pr(>|z|)
Location in garden sampled from:		
Border (baseline)		
+ compost bag	1.43 (0.31-7.40)	0.64
+ compost heap	1.61 (0.26-10.24)	0.61
+ manure bag	-[a]	0.99
+ manure bag + compost heap	-[a]	0.99
Compost bag	**15.70 (5.50-66.19)**	**<0.01**
Compost heap	**3.45 (1.93-6.40)**	**<0.01**
Manure bag	-[a]	0.99
Pot/planter	**2.42 (1.50-3.95)**	**<0.01**
+ compost bag	**7.07 (2.88-21.28)**	**<0.01**
+ compost bag + compost heap	2.14 (0.20-46.50)	0.53
+ compost bag + manure bag	-[a]	0.98
+ compost heap	-[a]	0.99

Table 4: Odds ratios, confidence intervals and p-values from logistic regression model using location in garden that sample was collected from as an explanatory variable for whether samples (*n* = 501) grew *Aspergillus fumigatus*.

Significant results (p <= 0.05) are highlighted in bold.

[a] Insufficient data to calculate odds ratio and confidence intervals.

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Environmental variable	Environmental variable	Pr(>|z|)
Location in garden sampled from:		
Border (baseline)		
+ compost bag	1.64 (0.08-10.32)	0.65
+ compost heap	-[a]	0.99
+ manure bag	-[a]	0.99
+ manure bag + compost heap	-[a]	0.99
Compost bag	**6.79 (3.25-14.37)**	**<0.01**
Compost heap	**4.74 (2.45-9.32)**	**<0.01**
Manure bag	-[a]	0.99
Pot/planter	**2.88 (1.52-5.53)**	**<0.01**
+ compost bag	**3.05 (1.22-7.26)**	**<0.01**
+ compost bag + compost heap	-[a]	0.99
+ compost bag + manure bag	-[a]	0.99
+ compost heap	-[a]	0.99

Table 5: Odds ratios, confidence intervals and p-values from logistic regression model using location in garden that sample was collected from as an explanatory variable for whether samples (*n* = 509) grew tebuconazole-resistant *Aspergillus fumigatus*.

Significant results (p <= 0.05) are highlighted in bold.

[a] Insufficient data to calculate odds ratio and confidence intervals.

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