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Citizen Science Surveillance of Triazole-Resistant *Aspergillus fumigatus* in United Kingdom Residential Garden Soils

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ABSTRACT Compost is an ecological niche for *Aspergillus fumigatus* due to its role as a decomposer of organic matter and its ability to survive the high temperatures associated with the composting process. Subsequently, composting facilities are associated with high levels of *A. fumigatus* spores that are aerosolized from compost and cause respiratory illness in workers. In the UK, gardening is an activity enjoyed by individuals of all ages, and it is likely that they are being exposed to *A. fumigatus* spores when handling commercial compost or compost they have produced themselves. In the present study, 246 citizen scientists collected 509 soil samples from locations in their gardens in the UK, from which were cultured 5,174 *A. fumigatus* isolates. Of these isolates, 736 (14%) were resistant to tebuconazole: the third most-sprayed triazole fungicide in the UK, which confers cross-resistance to the medical triazoles used to treat *A. fumigatus* lung infections in humans. These isolates were found to contain the common resistance mechanisms in the *A. fumigatus cyp51A* gene TR_{34}/L98H or TR_{46}/Y121F/T289A, as well as the less common resistance mechanisms TR_{34}, TR_{53}, TR_{46}/Y121F/T289A/S363P/I364V/G448S, and $(TR_{46})^2$/Y121F/M172I/T289A/G448S. Regression analyses found that soil samples containing compost were significantly more likely to grow tebuconazole-susceptible and tebuconazole-resistant *A. fumigatus* strains than those that did not and that compost samples grew significantly higher numbers of *A. fumigatus* than other samples.

IMPORTANCE The findings presented here highlight compost as a potential health hazard to individuals with predisposing factors to *A. fumigatus* lung infections and as a potential health hazard to immunocompetent individuals who could be exposed to sufficiently high numbers of spores to develop infection. Furthermore, we found that 14% of *A. fumigatus* isolates in garden soils were resistant to an agricultural triazole, which confers cross-resistance to medical triazoles used to treat *A. fumigatus* lung infections. This raises the question of whether compost bags should carry additional health warnings regarding inhalation of *A. fumigatus* spores, whether individuals should be advised to wear facemasks while handling compost, or whether commercial producers should be responsible for sterilizing compost before shipping. The findings support increasing public awareness of the hazard posed by compost and investigating measures that can be taken to reduce the exposure risk.

KEYWORDS DNA sequencing, drug resistance mechanisms, environmental microbiology, molecular genetics, mycology, public health

The fungus *Aspergillus fumigatus* plays an important role in the environment as a decomposer, recycling nutrients from decaying plant matter into the soil. This highly sporulating mold is commonly found in woodchip piles and compost from

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household waste, sewage, sludge, and moldy hay (1), where its thermotolerance enables it to proliferate during the thermogenic phase of composting when temperatures reach 40 to 60°C (2). The small size of *A. fumigatus* spores (2 to 3 μm), and their hydrophobicity means they are easily aerosolized and transported on air currents, making *A. fumigatus* a globally ubiquitous fungus (3). Exposure to this mold is medically important, and it is estimated that humans inhale several hundred *A. fumigatus* spores per day (4), which can trigger an immunoinflammatory response resulting in severe asthma with fungal sensitization (SAFS) or allergic bronchopulmonary aspergillosis (ABPA) (5). The size of the spores allows them to bypass mucociliary clearance in the lung (6), whereupon they must then evade clearance by the host innate and adaptive immune responses (7). If they survive, germinated spores establish in lung cavities, where they can eventually cause chronic pulmonary aspergillosis (CPA). CPA affects apparently immunocompetent individuals with an existing lung condition, such as ABPA, chronic obstructive pulmonary disease, tuberculosis, or lung cancer, or an underlying immune dysfunction due to diabetes, rheumatoid arthritis, or alcoholism (8). If the host immune system is unable to prevent spores from entering the bloodstream, then invasive aspergillosis (IA) develops, which is a life-threatening infection associated with ~58% survival (9). Individuals who are immunocompromised due to treatment with immunosuppressants, chemotherapy, or HIV/AIDS infection are at greatest risk of IA (9). Furthermore, individuals admitted to intensive care units with severe influenza infection are at risk of developing influenza-associated pulmonary aspergillosis, which is associated with increased mortality (10). A similar disease is now being observed for COVID-19-associated pulmonary aspergillosis in individuals with severe COVID-19 infection (11). It was estimated that in the UK in 2011 there were ~178,000 individuals living with ABPA, 3,600 with CPA, and 2,900 with IA, plus an additional 377 to 1,345 cases of IA in critical care patients (12). The number of patients in the UK presenting with infections that are resistant to one or more of itraconazole (ICZ), voriconazole (VCZ), and posaconazole (PCZ)—the frontline triazole drugs for treating aspergillosis—increased from 3 to 7% between 1999 and 2001 to 14 to 20% between 2007 and 2009 (13). Triazole-resistant infections are associated with treatment failure, salvage therapy with more toxic antifungals and increased case fatality rates (CFRs), with CFRs up to 88% reported for triazole-resistant IA (14).

Triazole resistance is most commonly caused by polymorphisms in the *cyp51A* gene, which results in increased production of, or configurational changes in, lanosterol-14a-demethylase, an enzyme involved in ergosterol biosynthesis and the binding target of triazole drugs. An environmental route for the acquisition of triazole-resistant infections has been proposed due to the increase of infections caused by *A. fumigatus* isolates with a tandem repeat (TR) in the promoter region of *cyp51A* coupled with single-nucleotide polymorphisms in the coding region leading to amino acid substitutions in the protein, which are frequently recovered from air and soil samples globally (15). This is likely due to the use of the fungicides epoxiconazole, tebuconazole, propiconazole, difenoconazole, and bromuconazole, which have similar molecular structures to the medical triazoles and show cross-resistance (16). In 2008, these were the second, third, sixth, ninth, and seventeenth most sprayed triazoles in agriculture in the UK, respectively (17). In agriculture, triazoles are applied to wheat, beans, carrots, oilseed rape, soft fruits and vines; in horticulture, they are used to sterilize bulbs and to control fungal diseases in lawns and ornamentals; and in industry they are used as wood preservatives and antifouling agents in leather, paper, textiles, paints, and adhesives (17).

The UK government is committed to reducing carbon dioxide emissions by diverting waste from landfill and incineration to composting (18), and compost features in the government's Food 2030 strategy for improving the productive capacity of soil (19). Compost producers accept input material from agriculture, horticulture, forestry, wood, and paper processing, leather and textile industries, and household and garden waste, which are highly likely to contain triazole residues. In 2007, 90% of composting facilities in the UK produced compost in open windrows (20); where organic waste is shredded, mixed, and

TABLE 1 Breakdown of the soil samples collected[a]

Location in garden where the soil sample was collected[b]	No. of soil samples	No. of samples that grew:		No. of *A. fumigatus* isolates grown	Avg no. of *A. fumigatus* isolates (CFU/g)	No. of tebuconazole-resistant *A. fumigatus* isolates grown (% *A. fumigatus* isolates)	Avg no. of tebuconazole-resistant *A. fumigatus* isolates (CFU/g)
		A. fumigatus (%)	Tebuconazole-resistant *A. fumigatus* (%)				
B	206	99 (48)	19 (9)	1,009	204	121 (12)	127
+ CB	7	4 (57)	1 (14)	56	280	8 (14)	160
+ CH	5	3 (60)	0 (0)	44	293	0 (0)	0
+ MB	1	0 (0)	0 (0)	0	0	0 (0)	0
+ MB + CH	1	1 (100)	0 (0)	1	20	0 (0)	0
CB	49	44 (90)	20 (41)	993	451	137 (14)	137
CH	80	58 (73)	27 (34)	1,464	505	289 (20)	214
MB	1	1 (100)	0 (0)	30	600	0 (0)	0
PP	115	79 (69)	26 (23)	1,005	254	130 (13)	98
+ CB	38	33 (87)	8 (21)	529	321	51 (15)	128
+ CB + CH	3	2 (67)	0 (0)	21	210	0 (0)	0
+ CB + MB	2	2 (100)	0 (0)	4	40	0 (0)	0
+ CH	1	1 (100)	0 (0)	18	360	0 (0)	0
Total	509	327 (64)	101 (20)	5,174	316	736 (14)	145

[a]The table shows a breakdown of the number of soil samples collected, the number and percentage of soil samples that grew tebuconazole-susceptible and tebuconazole-resistant *Aspergillus fumigatus*, the numbers of tebuconazole-susceptible and tebuconazole-resistant *A. fumigatus* isolates grown, and the average CFU/g across samples that grew tebuconazole-susceptible and tebuconazole-resistant *A. fumigatus* by the location(s) in the garden where the soil sample was collected.
[b]B, border; CB, compost bag; CH, compost heap, MB, manure bag; PP, pot/planter.

placed in uncovered rows that are turned regularly during the composting process to improve oxygenation of the waste and to distribute heat and moisture. Composting facilities are known to produce large numbers of *A. fumigatus* spores (20–27), with resulting negative health impacts on compost handlers (28–36), and there is evidence from the Netherlands that composting material also produces large numbers of triazole-resistant spores (37, 38). In 2017, UK households spent approximately £450 million on compost (39) and apply it more liberally to their gardens at 300 tonnes per hectare (t/ha) than the 50 t/ha applied to agricultural land (40). Furthermore, more than a third of households with access to a garden report composting their garden and/or kitchen waste (41). This means that a substantial proportion of the UK population is handling compost on a regular basis, with potential exposure to high levels of *A. fumigatus* spores that may have developed triazole resistance from composts that contain triazole residues. Indeed, there have been reports of hypersensitivity pneumonitis (42) and IA (43–47) in apparently immunocompetent individuals following gardening activities; however, no clinical links following exposure to triazole resistant spores have been documented.

The aims of this study were to (i) determine the numbers of triazole-susceptible and -resistant *A. fumigatus* spores in soil samples collected from residential gardens in the UK, (ii) characterize the *cyp51A* polymorphisms responsible for resistance, and (iii) find environmental variables associated with the presence/numbers of *A. fumigatus* spores in soil samples. In order to simultaneously sample a wide range of UK gardens, we were assisted by a network of citizen-scientists trained in the collection of samples that may contain *A. fumigatus*. Our aim was to ascertain whether gardening activities may lead to exposure to triazole-resistant genotypes of this mold that could present a risk to susceptible individuals. Based on our findings, we present thoughts on how these exposure risks in susceptible individuals might be mitigated.

RESULTS

Tebuconazole-susceptible and tebuconazole-resistant *A. fumigatus* in soil samples. Of the 509 soil samples collected, 327 (64%) samples between them grew 5,174 *A. fumigatus* isolates and 101 (20%) samples grew 736 tebuconazole-resistant isolates (Table 1). Most of the samples (n = 451; 89%) were assigned a singlelocation in the

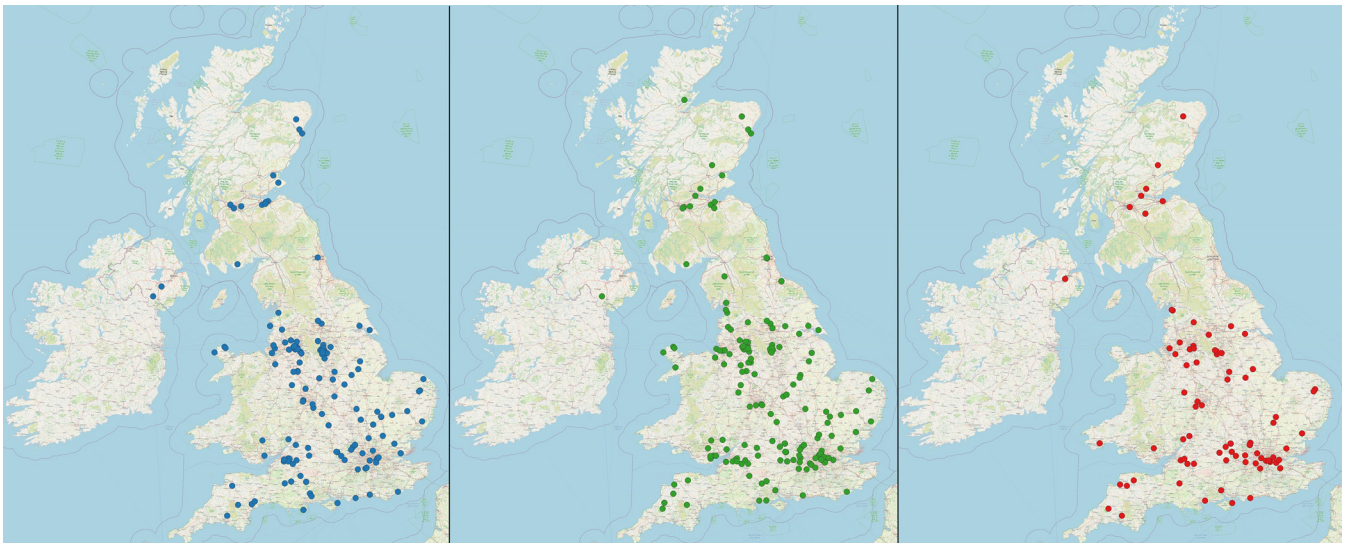

FIG 1 Geographic locations in the UK that soil samples were collected from by citizen scientists. Blue dots indicate samples that did not grow *Aspergillus fumigatus*, green dots indicate samples that grew *Aspergillus fumigatus*, and red dots indicate samples that grew tebuconazole-resistant *A. fumigatus*.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

garden where they were collected, whereas the remainder were assigned multiple locations. These multiple locations occurred when a border or pot/planter had recently been topped up with manure or compost. The concentration of spores and mycelial fragments averaged across the samples that grew *A. fumigatus* was 316 CFU/g, which ranged from 0 CFU/g in the sample collected from a border plus manure bag to 600 CFU/g in the sample collected from a manure bag. The concentration of spores and mycelial fragments averaged across the samples that grew tebuconazole-resistant *A. fumigatus* was 146 CFU/g, which ranged from 0 CFU/g in samples collected from several garden locations to 214 CFU/g in samples collected from compost heaps. Figure 1 shows the geographical locations in the UK where soil samples were collected.

Cyp51A polymorphisms in tebuconazole-resistant *A. fumigatus* isolates. Of the 736 tebuconazole-resistant *A. fumigatus* isolates, 93 (13%) failed to regrow from refrigerated storage for cryopreservation and DNA extraction. In the 643 isolates that regrew, TR_{34}/L98H was detected in 542 (85%), TR_{46}/Y121F/T289A was detected in 16 (3%), TR_{53} was detected in 2, and $(TR_{48})^2$/Y121F/M172I/T289A/G448S was detected in 1 sample, and no *cyp51A* polymorphisms were detected in 27 (4%) isolates. A total of 14 isolates failed to sequence with the *cyp51A* promoter and coding region primers, and beta-tubulin sequencing confirmed their identities as *A. fischeri* ($n = 8$), *A. fumigatus* ($n = 2$), *A. oerlinghausenensis* ($n = 3$), and unknown ($n = 1$). Uncommon polymorphisms detected were TR_{34} without accompanying amino acid substitutions in three isolates, $(TR_{34})^2$/L98H in one isolate, and $(TR_{130})^3$/D430G in four isolates. The remaining isolates contained one or more amino acid substitutions in cyp51A, with or without accompanying TRs (Table 2). Further details regarding the tebuconazole-resistant *A. fumigatus* isolates can be found in Table S1 in the supplemental material.

Environmental variables influencing growth and numbers of *A. fumigatus* colonies. (i) Growth of *A. fumigatus* from soil samples. Eight samples were excluded from the logistic regression with growth of *A. fumigatus* as the outcome, which left 501 samples in the analysis. These samples were excluded because the Sabouraud dextrose agar (SDA) plates were too contaminated to determine the presence of *A. fumigatus*. The location in the garden where the soil sample was collected was the only variable that significantly affected whether a sample grew *A. fumigatus* ($\chi^2 = 67.3$, df = 12, $P < 0.01$). The odds ratios and *P* values from the logistic regression model are shown in Table 3. Samples collected from a compost bag, compost heap, pot/planter, and

TABLE 2 *cyp51a* polymorphisms for the 636 tebuconazole-resistant *Aspergillus fumigatus* isolates grouped by the garden location where they were collected

Tandem repeat in *cyp51A* promoter region	Amino acid substitution(s) in *cyp51A*	No. of samples based on the location where the garden soil sample was collected[a]						Total	Medical triazole susceptibility (reference)
		B	B+CB	CB	CH	PP	PP+CB		
–	–	3			1	23		27	
–	C270R					1		1	
–	I242V					5		5	63
TR_{34}	–				1	1	1	3	57
TR_{34}	L98H	82	7	117	237	65	34	542	71
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	55
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	52
$(TR_{46})^2$	Y121F/M172I/T289A/G448S				1			1	38
TR_{53}	–	1			1			2	59
$(TR_{130})^3$	D430G				4			4	72
Failed to sequence	Failed to sequence[b]	3			3	8		14	
Total		91	8	124	276	107	37	643	

[a]B, border; CB, compost bag; CH, compost heap, MB, manure bag; PP, pot/planter.
[b]Samples that failed to amplify with the *cyp51A* promoter and coding region primers were sequenced using beta-tubulin primers for fungal identification.

pot/planter plus compost bag had significantly increased odds of growing *A. fumigatus* ($P < 0.01$) compared to samples collected from a border. There were no significant changes in odds of growing *A. fumigatus* from other sampling locations.

(ii) Number of *A. fumigatus* colonies grown from soil samples. The first negative binomial regression was run on the 335 samples that grew *A. fumigatus*. The only variable found to significantly affect the number of *A. fumigatus* colonies grown from a sample was garden location from which the sample was collected ($\chi^2 = 50.8$, df = 11, $P < 0.01$). In the regression model, samples collected from compost bag ($P < 0.01$), compost heap ($P < 0.01$), and pot/planter plus compost bag ($P = 0.02$) grew significantly more *A. fumigatus* colonies than samples collected from borders. Samples collected from a pot/planter plus compost bag plus manure bag grew fewer *A. fumigatus* colonies than samples collected from borders, although this reduction was marginally significant ($P = 0.05$).

(iii) Growth of tebuconazole-resistant *A. fumigatus* from soil samples. All 509 soil samples were included in the logistic regression with growth of tebuconazole-resistant *A. fumigatus* as the outcome. The only variable found to significantly affect whether a sample grew tebuconazole-resistant *A. fumigatus* was garden location from which the sample was collected ($\chi^2 = 43.0$, df = 12, $P < 0.01$). The odds ratios and P values from the logistic regression model are shown in Table 4. Samples collected from a compost

TABLE 3 Odds ratios, confidence intervals, and *P* values from a logistic regression model using location in garden that sample was collected from as an explanatory variable for whether samples ($n = 501$) grew *Aspergillus fumigatus*[a]

Garden location sampled	OR (95% CI)[b]	Pr(>|z|)
Border (baseline)		
+ compost bag	1.43 (0.31–7.40)	0.64
+ compost heap	1.61 (0.26–10.24)	0.61
+ manure bag	–	0.99
+ manure bag + compost heap	–	0.99
Compost bag	**15.70 (5.50–66.19)**	**<0.01**
Compost heap	**3.45 (1.93–6.40)**	**<0.01**
Manure bag	–	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	–	0.98
+ compost heap	–	0.99

[a]Significant results ($P \leq 0.05$) are highlighted in boldface.
[b]OR, odds ratio. –, Insufficient data to calculate the odds ratio and confidence intervals (CI).

bag, compost heap, pot/planter, and pot/planter plus compost bag had significantly increased odds of growing tebuconazole-resistant *A. fumigatus* ($P < 0.01$) compared to samples collected from a border. There were no significant changes in the odds of growing tebuconazole-resistant *A. fumigatus* from other sampling locations.

(iv) Number of tebuconazole-resistant *A. fumigatus* colonies grown from soil samples. The second negative binomial regression was run on the 101 samples that grew tebuconazole-resistant *A. fumigatus*. None of the environmental variables were found to have a significant effect on the outcome.

DISCUSSION

In this study, 5,174 *A. fumigatus* isolates were cultured from 509 soil samples collected by 249 citizen scientists from their gardens across the UK (48). Of these soil samples, 327 (64%) grew *A. fumigatus* isolates, and 101 (20%) grew isolates that were resistant to tebuconazole at a concentration of 6 mg/L. The percentage of soils that grew *A. fumigatus* in this study was lower than the 78% of soils collected by Sewell et al. from several sites across South West England, including parks, cemeteries, public gardens, flower beds outside hospitals, a lavender farm, a forest, and farmland (49).

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 ($n = 509$) grew tebuconazole-resistant *Aspergillus fumigatus*[a]

Garden location sampled	OR (95% CI)[b]	Pr(>|z|)
Border (baseline)		
+ compost bag	1.64 (0.08–10.32)	0.65
+ compost heap	–	0.99
+ manure bag	–	0.99
+ manure bag + compost heap	–	0.99
Compost bag	**6.79 (3.25–14.37)**	**<0.01**
Compost heap	**4.74 (2.45–9.32)**	**<0.01**
Manure bag	–	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	–	0.99
+ compost bag + manure bag	–	0.99
+ compost heap	–	0.99

[a]Significant results ($P \leq 0.05$) are highlighted in boldface.
[b]OR, odds ratio. –, Insufficient data to calculate the odds ratio and confidence intervals (CI).

However, the percentage of soils in this study that grew tebuconazole-resistant *A. fumigatus* isolates was greater than the 6% of soils in Sewell et al. that grew *A. fumigatus* with increased MICs to ICZ, VCZ, and/or PCZ (49). Of the 5,174 *A. fumigatus* isolates cultured in this study, 736 (14%) were resistant to tebuconazole, which is greater than the 6% prevalence of triazole-resistant *A. fumigatus* reported by Tsitsopoulou et al. from urban and rural soils in South Wales (50) and the absence of triazole-resistance detected by van der Torre et al. in isolates cultured from soils adhered to vegetables grown in the UK (51). This prevalence of 14% is also greater than the 9% in experimental cropland and 12% in commercial wheat fields in the UK reported by Fraaije et al. (52); however, it is less than the 37% prevalence in isolates cultured from flower bulbs bought from a garden center in Dublin reported by Dunne et al. (53). In this study, the average concentration of *A. fumigatus* from positive soil samples was 316 CFU/g, which is higher than the 43.5 CFU/g in agricultural soils and 106 CFU/g in urban soils from Greater Manchester reported by Bromley et al. (54) and considerably higher than the 0 to 10 CFU/g reported from woodlands, grass verges, experimental cropland, and commercial wheat fields across the UK reported by Fraaije et al. (52). Given that *A. fumigatus* is often considered to be ubiquitous in the environment, it is intriguing that 36% of the soil samples collected in this study did not grow this mold. We speculate that *A. fumigatus* spores and mycelial fragments in garden soils are killed by triazole residues from dipped bulbs (53), for example, if they have not developed triazole resistance. It is also possible that *A. fumigatus* is outcompeted by other microbes, especially in soils that have not experienced the high temperatures that are associated with composting.

Of the 736 *A. fumigatus* isolates that grew on tebuconazole at 6 mg/L, 93 (13%) did not regrow from short-term storage in the fridge, which left 643 (87%) isolates for sequencing of the *cyp51A* promoter and gene coding regions. Similar to existing UK studies (49, 50, 54), the predominant mutation identified in this study was TR_{34}/L98H ($n = 535$; 73%). Of these isolates, 22 had amino acid substitutions in cyp51A in addition to L98H. Six isolates had T289A, I364V, and G448S amino acid substitutions, in addition to TR_{34}/L98H, which has been previously detected in Korea in a patient with IA (55) and in Japan on tulip bulbs imported from The Netherlands (56). TR_{68}/L98H was detected in one isolate, which was found to be two repeats of the 34-bp insert, and in three isolates TR_{34} was detected without any accompanying amino acid substitutions, which was first detected in an environmental isolate collected from Scotland (57). TR_{46}/Y121F/T289A was detected in 16 (2%) isolates and was accompanied by S363P, I364V, and G448S in four additional isolates, a combination reported from The Netherlands in 2018 (52). Additional polymorphisms detected in this study included TR_{53}, which has been previously reported from flower fields in Colombia (58) and from a patient with multiple-azole-resistant *A. fumigatus* osteomyelitis in The Netherlands (59), and TR_{92}/Y121F/M172I/T289A/G448S, which has been previously detected in flower bulb waste in The Netherlands (38) and is two repeats of the 46-bp insert. There were 33 (4%) isolates in this study that did not contain any TRs: five contained I242V, one contained C270R, and 27 had no amino acid substitutions in *cyp51A*. I242V is the only single *cyp51A* amino acid substitution detected in this study to have been reported in studies summarizing *cyp51A* polymorphisms (60–63), which may suggest these polymorphisms occurred *in situ*. The 28 isolates that did not contain any *cyp51A* polymorphisms may well be using non-*cyp51A* mechanisms for triazole resistance, such as the overexpression of efflux pumps, *cyp51B* overexpression, cholesterol import, or *hapE* mutation, which were not explored in this study (64).

The only environmental variable measured in this study that was found to have a significant effect on whether a sample grew *A. fumigatus* or on the numbers of *A. fumigatus* grown was the garden location from which the sample was collected. The greatest concentration of *A. fumigatus* was cultured from a bag of manure at 600 CFU/g, followed by homemade compost heap samples at 505 CFU/g, commercial compost bag samples at 451 CFU/g, and pot/planters containing commercial compost at 321 CFU/g. Soil samples that did not contain compost grew fewer *A. fumigatus* isolates: 254 CFU/g

from pot/planters and 204 CFU/g from borders. Similar observations were made for tebuconazole-resistant *A. fumigatus*, with concentrations of 128 to 289 CFU/g recorded for samples containing compost and of 98 to 127 CFU/g for samples without compost. As citizen scientists were only asked to indicate one garden location from which the soil sample was collected, it is possible that the concentrations of *A. fumigatus* spores from borders and pot/planters were inflated by the recent addition of compost that was not indicated on the questionnaire. In the regression models, soils collected from commercial compost bags, homemade compost heaps, pot/planters, and pot/planters plus commercial compost had significantly greater odds of growing *A. fumigatus* and tebuconazole-resistant *A. fumigatus* ($P < 0.01$ for all associations) compared to soil samples collected from borders. Furthermore, samples collected from commercial compost bags, homemade compost heaps, and pot/planter plus commercial compost grew significantly more *A. fumigatus* colonies compared to samples collected from borders. No association was found for garden locations sampled from and numbers of tebuconazole-resistant *A. fumigatus*.

Several existing studies have looked for triazole-resistant *A. fumigatus* specifically in compost in the UK and globally, and the findings have been highly variable. Tsitsopoulou et al. collected 11 compost samples from agricultural fields, a horticultural nursery and public areas across South Wales that grew 10 *A. fumigatus* isolates in all—none of which were triazole resistant (50). Dunne et al. do not report how many samples they collected from commercial compost bought from a garden center in Dublin or how many *A. fumigatus* were cultured from these samples; only that one isolate was triazole-resistant (53). Sewell et al. collected two samples from a compost heap in London that, combined with three samples collected from a flower bed ~500 m away, gave a 60% prevalence of triazole-resistant *A. fumigatus* (49). Pugliese et al. sampled from composting orange peel in Italy and found *A. fumigatus* concentrations of 8.8×10^3 CFU/g at the start of the process rising to 605.7×10^3 CFU/g by the end, and yet none of the 30 isolates selected for susceptibility testing were triazole resistant (65). Santoro et al. sampled from 11 green and brown composts across Spain, Hungary, and Italy and found concentrations of *A. fumigatus* ranging from 100 to 10.6×10^3 CFU/g; none of the 30 isolates selected for susceptibility testing were triazole resistant (66). Ahangarkani et al. screened isolates cultured from 300 compost samples collected in Iran and detected 57 isolates with elevated MICs to ICZ and VCZ (67). Zhang et al. collected 114 samples from a plant waste stockpile over 16 months in the Netherlands and detected $>10^3$ *A. fumigatus* CFU/g in 74% of samples, with the prevalence of triazole-resistant *A. fumigatus* averaging 50% across all samples (37). Also in The Netherlands, Schoustra et al. found concentrations of triazole-resistant *A. fumigatus* of 200 CFU/g in household green waste, $(1.5 \text{ to } 1.8) \times 10^3$ CFU/g in compost heaps in residential gardens, up to 2.3×10^5 CFU/g in flower bulb waste, and up to 8.4×10^4 CFU/g in organic waste from landscaping (38).

The key findings of this study are that 64% of soil samples collected from residential gardens in the UK grew *A. fumigatus* and that 20% of samples grew tebuconazole-resistant *A. fumigatus*. This means that individuals are very likely to be exposed to both *A. fumigatus* and triazole-resistant *A. fumigatus* spores that are aerosolized from soil when they are undertaking gardening activities (43–47). Although this study has not undertaken susceptibility testing for the tebuconazole-resistant *A. fumigatus* isolates against medical triazoles, the most commonly detected *cyp51A* polymorphisms TR_{34}/L98H and TR_{46}/Y121F/T289A are associated with elevated MICs to ICZ, PCZ, and VCZ (68). Furthermore, Hodiamont et al. reported a clinical isolate containing TR_{53} as being resistant to ICZ and VCZ, with reduced susceptibility to PCZ (59). This study also reports that the likelihood of being exposed to *A. fumigatus* and triazole-resistant *A. fumigatus* spores is significantly greater when handling commercial or homemade compost compared to soils in borders or pots/planters. The 14% prevalence of triazole resistance detected in garden soil samples in this study is higher than most existing studies that have sampled from rural and urban locations in the UK, which is likely being driven by the concentrated application of compost in residential settings. The National Aspergillosis Centre advises that people take care when

opening bags of compost and recommends wearing a facemask while doing so to avoid dust inhalation. Currently, the only health warning on commercial compost bags is for women to not handle compost without gloves if they are pregnant, presumably to avoid toxoplasmosis infection (69). The evidence presented here supports the recommendation for users to wear a mask while handling compost and the introduction of health warnings on bags of compost with regard to inhaling *A. fumigatus*. Measures could also be taken by compost producers to sterilize the composting before packaging, thereby killing viable *A. fumigatus* spores and eliminating the immediate hazard it poses to the user.

MATERIALS AND METHODS

Culturing *Aspergillus fumigatus* from residential garden soil samples. The soil samples from which *A fumigatus* isolates were cultured for this study were collected as part of a citizen science project undertaken in June 2019, which involved 246 volunteers in the UK collected a total of 509 soil samples from different locations in their gardens (48). Participants indicated on a questionnaire whether samples were collected from a border, pot or planter, compost heap, bag of manure, or bag of compost. Upon receipt, 2 g of each soil sample was suspended in 8 mL of buffer (0.85% NaCl and 0.01% Tween 20 in distilled water), shaken vigorously, and left to settle for 30 min. No adjustment was made for the moisture content of the soil when weighing it out. One 200-μL aliquot from the surface of the buffer was spread onto a plate containing SDA, penicillin (200 mg/L), and streptomycin (400 mg/L) and a second aliquot of 200 μL was spread onto a plate containing SDA, penicillin (200 mg/L), streptomycin (400 mg/L), and tebuconazole (6 mg/L). The concentration of 6 mg/liter tebuconazole was chosen after testing the growth of 30 isolates with known *CYP51A* mutations on SDA supplemented with 0, 4, 6, 8, and 16 mg/L tebuconazole. The only concentration that showed no growth of any isolates without *CYP51A* mutations and partial or full growth of all isolates with *CYP51A* mutations was 6 mg/L. Both plates were incubated at 37°C for 48 h, the number of colonies that morphologically resembled *A. fumigatus* on each plate recorded, and the colonies growing on the plate containing tebuconazole were picked into tubes containing mold preservation solution (0.2% agar and 0.05% Tween 20 in deionized water) and stored at 4°C. These isolates were subsequently cryopreserved in 50% glycerol solution and were DNA extracted as detailed by Boyle et al. (70).

Sequencing of *A. fumigatus cyp51A* gene. The promoter region of *cyp51A* was amplified using forward primer 5′-GGACTGGCTGATCAAACTATGC-3′ and the reverse primer 5′-GTTCTGTTCGGTTCCAAAGCC-3′ and the following PCR conditions: 95°C for 5 min; 30 cycles of 98°C for 20 s, 65°C for 30 s, and 72°C for 30 s; followed by 72°C for 5 min. The PCR volume used was 50 μL: 10 μL of FIREPol DNA polymerase (Solis Biodyne, Estonia), 10 μL of forward primer (1.5 μM; Invitrogen, USA), 10 μL of reverse primer (1.5 μM; Invitrogen, USA), 18 μL of nuclease-free water (Merck, Germany), and 2 μL of DNA. Amplicons were visualized by gel electrophoresis, and samples with visible bands were sent for sequencing using the forward primer. The coding region of *cyp51A* was amplified using the forward primer 5′-ATGGTGCCGATGCTATGG-3′ and the reverse primer 5′-CTGTCTCACTTGGATGTG-3′ and the following PCR conditions: 94°C for 2 min; 35 cycles of 94°C for 30 s, 60°C for 45 s, and 72°C for 45 s; followed by 72°C for 5 min. The PCR volume used was 50 μL: 0.2 μL of Q5 high-fidelity DNA polymerase (New England Biolabs, UK), 10 μL of Q5 reaction buffer (5×; New England Biolabs, UK), 0.5 μL of deoxynucleotide (dNTP) solution mix (40 μM; New England Biolabs, UK), 1 μL of forward primer (10 μM; Invitrogen, USA), 1 μL of reverse primer (10 μM; Invitrogen, USA), 35.3 μL of nuclease-free water (Merck, Germany), and 2 μL of DNA. Amplicons were visualized by gel electrophoresis, and samples with visible bands were sent for sequencing using the Sanger chain termination method in two segments using the primers 5′-TACGTTGACATCATCAATCAG-3′ and 5′-GATTCACCGA ACTTTCAAGGCTCG-3′. Sequences were aligned using Molecular Evolutionary Genetics Analysis (MEGA) software (Penn State University).

Identification of isolates. For isolates that failed to sequence using the primers for the promoter and coding regions of *cyp51A*, part of the beta-tubulin gene was sequenced using the forward primer 5′-AATTGGTGCCGCTTTCTGG-3′ and the reverse primer 5′-AGTTGTCGGGACGGAATAG-3′ and the following PCR conditions: 94°C for 3 min; 30 cycles of 94°C for 15 s, 55°C for 30 s, and 68°C for 30 s; followed by 68°C for 3 min. Amplicons were visualized by gel electrophoresis, and samples with visible bands were sent for sequencing using the forward primer. The Basic Local Alignment Search Tool (BLAST) was used to align the sequences to those in the National Center for Biotechnology Information (NCBI, Bethesda, MD) to identify the isolate.

Environmental variables that may influence growth of *Aspergillus fumigatus*. Table 5 details the environmental variables that were ascertained for the locations in the UK where soil samples were collected, the date the sampling occurred, and the sources from which the data were obtained.

Generalized linear models. Generalized linear models (GLMs) were run using R version 4.0.0 to find associations between the environmental variables in Table 5 and (i) the likelihoods of a sample growing susceptible or triazole-resistant *A. fumigatus* and (ii) the number of susceptible or triazole-resistant *A. fumigatus* colonies grown from a sample. Growth of triazole-susceptible or triazole-resistant *A. fumigatus* from a sample was categorized as 0/1 and logistic regressions ("glm" function; family = "binomial") were performed. The numbers of susceptible and triazole-resistant *A. fumigatus* colonies grown from samples were overdispersed; therefore, negative binomial regressions (library "MASS"; "glm.nb" function) were performed. Environmental variables were included in the regression model based on a significant improvement on the null model, as determined by analysis of variance (ANOVA) using chi-squared test. Results

TABLE 5 Environmental variables obtained for soil sampling locations and dates, as well as source locations

Environmental variables ascertained for sampling date and location	Source of information (references)
Garden location where soil sample was collected	Citizen scientist
Date the sample was collected	Citizen scientist
Maximum daily temp at sampling location on sampling date	Met Office HadUK-Grid dataset (73)
Land cover classification of sampling location	UKCEH Land Cover Map 2019 (74)
Urban or rural classification of sampling location	Calculated from land cover classification
Percent arable land in 2-km buffer surrounding sampling location	Calculated from UKCEH Land Cover Map 2019 using QGIS 3.16.4 (75)
Distance of sampling location to nearest composter with open windrow or outdoor activity	Composter locations were obtained from Environment Agency, Scottish Environment Agency (SEPA) website (76), Natural Resources Wales website (77), and Northern Ireland Environment Agency website (78). Distances were calculated using package "geosphere" in R version 4.0.0 (79).

were considered significant when $P \leq 0.05$. The regression model with the best fit was chosen based on a reduced Akaike information criterion (AIC) score and a significant improvement on the null model.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

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The authors have no competing interests to declare.

J.M.G.S., A.C.S., and M.C.F. conceptualized the study. A.A. and P.S.D. contributed experimental techniques. J.M.G.S. and R.C. processed samples. J.M.G.S. and C.B.U. analyzed the data. J.M.G.S. drafted the original manuscript, which C.B.U., A.C.S., and M.C.F. reviewed and edited.

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