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# Citizen science identifies the effects of nitrogen dioxide and other environmental drivers on tar spot of sycamore[☆]

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ABSTRACT

Elevated sulphur dioxide ($SO_2$) concentrations were the major cause of the absence of symptoms of tar spot (*Rhytisma acerinum*) of sycamore (*Acer pseudoplatanus*), in urban areas in the 1970s. The subsequent large decline in $SO_2$ concentrations has not always been accompanied by increased tar spot symptoms, for reasons that have remained unresolved. We used a large citizen science survey, providing over 1000 records across England, to test two competing hypotheses proposed in earlier studies. We were able to demonstrate the validity of both hypotheses; tar spot symptoms were reduced where there were fewer fallen leaves as a source of inoculum, and elevated nitrogen dioxide concentrations reduced tar spot symptoms above a threshold concentration of about 20 $\mu g\ m^{-3}$. Symptom severity was also lower at sites with higher temperature and lower rainfall. Our findings demonstrate the power of citizen science to resolve competing hypotheses about the impacts of air pollution and other environmental drivers.



## 1. Introduction

Citizen science is a research method that is increasingly being used to capture environmental data (Silvertown, 2009). It can be broadly characterised as a method that uses volunteers in the collection and/or analysis of scientifically useful data, particularly those related to environmental observations and biological monitoring (Roy et al., 2012). Citizen science allows phenomena to be studied at a geographical and temporal range that is normally outside the scope of what is typically achieved by individual researchers (Dickinson et al., 2010). It has proved successful in many instances: for example, Bonney et al. (2009) cite several examples of the role that citizen science has played in a variety of ornithological studies in North America, and citizen science is having an impact on tree health monitoring in the UK (e.g. Pocock and Evans, 2014). Despite the success of these projects, there are concerns regarding the quality of data derived by volunteers, although it is acknowledged that, with careful project design and data handling, these concerns can be overcome (Bonney et al., 2009). Nonetheless, citizen science is recognised as a powerful instrument, not only for advancing research, but also as a tool for engaging the public in science and improving their understanding of scientific methods (Dickinson et al., 2012).

The concept of using the presence or frequency of biological organisms as an indicator of air quality originates in the 19th century, with the work of Nylander (1866) on lichens in Paris. Although there are examples from the past of using simplified bioindicator methods to allow citizen science surveys of air pollution (e.g. Gilbert, 1974), there has been little recent use of this approach. One specific bioindicator of the presence of high levels of air pollution is visual symptoms of fungal infections on leaves, including the presence of leaf yeasts (Dowding and Richardson, 1990). The most well-known of these fungal bioindicators is *Diplocarpon rosae*, for which Saunders (1966) found a relationship between sulphur dioxide ($SO_2$) concentrations and the intensity of leaf infection, which was subsequently confirmed by fumigation experiments. A related bioindicator of elevated $SO_2$ concentrations is *Rhytisma acerinum*, which forms black typically circular stromata (tar spots) on the upper surface of leaves of *Acer pseudoplatanus* (Jones, 1925). *Rhytisma acerinum* overwinters in fallen leaves, and re-infection of new leaves occurs through ascospore release from these fallen leaves in the following spring, typically in cool wet

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conditions when this infection stage may be particularly sensitive to air pollution. Formation of the typical black spots takes about another two months after infection, and symptoms are most apparent in late summer.

Vick and Bevan (1976) reported a survey in and around Liverpool in which tar spot symptoms were only found at sites with annual mean or early summer $SO_2$ concentrations below 80–90 $\mu g\ m^{-3}$, a threshold similar to that reported by Saunders (1966) from both field surveys and controlled experimental exposures for *Diplocarpon rosae*. Based on further transplant experiments, Bevan and Greenhaigh (1976) suggested that $SO_2$ inhibited the germination or penetration stage of leaf infection during the spring. This study also introduced the use of a tar spot index, defined as the number of spots per 100 $cm^2$ of leaf area, which has been adopted in subsequent studies. In the UK, as in many areas of Europe and North America, $SO_2$ concentrations have fallen dramatically over recent decades, to levels at which no effects on the presence of tar spot symptoms would be expected. This was partly confirmed by a study in 1999 by Jarraud (2000), who resurveyed a number of sites in South Yorkshire where $SO_2$ concentrations were high and tar spot was reported to be absent in the early 1970s; at all but one site, tar spot was now present, an effect that could be attributed to the much lower $SO_2$ concentrations.

However, two studies have reported a continued low tar spot index in urban areas of the UK, despite the much lower $SO_2$ concentrations. Leith and Fowler (1988) described an absence of tar spot symptoms on *Acer pseudoplatanus* from the city centre of Edinburgh, which they attributed to a lack of inoculum caused by active clearing of leaves from urban parks and city streets, rather than air pollution. They confirmed this by demonstrating that, when *Acer pseudoplatanus* seedlings and inoculum on fallen leaves were transplanted together to two urban and one rural sites, there was no significant difference in the tar spot index that developed at the three sites, implying that $SO_2$ levels did not affect the degree of tar spot development when inoculum was present.

Jarraud (2000) reported a large decrease in tar spot index along a gradient into London, but attributed this to elevated concentrations of nitrogen dioxide ($NO_2$), which, in contrast to $SO_2$, remained high in the city centre. He also carried out transplant experiments in which inoculum and *Acer pseudoplatanus* saplings from a single rural site were exposed together at sites along the pollution gradient. In contrast to Leith and Fowler (1988), a strong effect of decreasing tar spot index with increasing proximity to the city centre was found, confirming that an environmental factor (most likely air pollution) was responsible for the observed gradient in tar spot index, rather than the availability of inoculum.

Subsequently, there has been little further research to test these two competing hypotheses. A field survey in southern Poland (Kosiba, 2007) showed that the density of tar spots on maple leaves was lower at more polluted sites with high leaf contents of sulphur, nitrogen and heavy metals, while Kosiba (2009) showed that the occurrence of *Rhytisma acerinum* on *Acer platanoides* was a good discriminator of sites of high and low environmental status across Poland. Almost all previous studies of *Rhytisma acerinum* on *Acer pseudoplatanus* have focussed on individual cities, or polluted regions such as south Yorkshire and southern Poland, and have involved a limited number of sites. There has been no larger-scale survey which might allow the effect of different environmental drivers on the distribution of tar spot symptoms to be distinguished.

The major citizen science programme, Open Air Laboratories (OPAL), which began in December 2007, aims to get more people outdoors to explore the world around them while contributing environmental data for research. Through OPAL, the public were invited to participate in England-wide surveys on different environmental themes, including soil and earthworms (Bone et al., 2012), urban invertebrates (Bates et al., 2015), and climate (Fowler et al., 2013). The OPAL air survey comprised two activities related to bio-indicators of air pollution, and specifically nitrogen oxides and ammonia. The first activity asked participants to record the abundance of lichens on tree trunks and twigs; the results of the national data were reported by Seed et al. (2013), while Tregidgo et al. (2013) showed that the method could identify impacts on lichen communities close to major roads.

The second activity in the OPAL air survey, which is the focus of this paper, asked participants to record the symptoms of tar spot on *Acer pseudoplatanus* trees. Leaf symptoms on *Acer pseudoplatanus* are well suited to such a national survey as the species is widespread across the UK, and is a common tree species in cities, where air pollution levels are generally higher. Inclusion of *Rhytisma acerinum* in this major national citizen science survey allowed us to test two key hypotheses:-

1. The tar spot index on *Acer pseudoplatanus* is lower at sites with fewer fallen leaves to provide an overwintered source of infection for the next spring
2. The tar spot index on *Acer pseudoplatanus* is lower at sites with a high concentration of $NO_2$

In addition, we tested whether climatic factors were associated with the tar spot index.

## 2. Methods

### 2.1. Survey design

The OPAL Air Survey was disseminated throughout England from September 2009. Participants requested survey packs, which included instructions and a recording booklet, via the OPAL website or by directly contacting OPAL staff who were distributed across England. In the tar spot element of the survey, participants selected 2–4 *Acer pseudoplatanus* trees, with the aid of a tree identification guide, and recorded the trunk girth at 1 m above the ground. They estimated the number of fallen leaves beneath the tree using a scale of 0 (no leaves), 1 (a small amount of leaves) and 2 (lots of fallen leaves), aided by appropriate photographs. Participants then selected up to 10 leaves that they could reach, or which had fallen, recorded the number of tar spots present (including partial spots), and measured the width of each leaf at its widest point; this was used as a surrogate measurement of leaf area. They also recorded the type of location of the recorded trees (e.g. in a street, at the edge of a wood). Participants submitted their results using the OPAL website, where they could select their location using a Google Map application and the date on which they carried out the survey. A small number submitted their results by post.

### 2.2. Data collation and screening

A total of 859 surveys, containing data on 2501 trees and submitted between September 2009 and June 2014, were downloaded from the database for analysis. We removed data on 297 trees surveyed outside England, where the overall survey density was much lower, and a further 217 that did not have complete information on all the relevant parameters. A further 269 trees surveyed between February and July were then removed, since tar spots are unlikely to be fully developed and visible during this time period. Inspection of the remaining 1718 records suggested that there were a number of outliers which most likely were due to observer error. Based on our own observations of a range of *Acer pseudoplatanus* leaves, we removed 73 records with leaf widths outside the range of

7–30 cm, and with more than 100 recorded tar spots per leaf. From the remaining leaves, the average number of tar spots per centimetre of leaf width, hereafter referred to as tar spot index, was calculated for each tree. In order to reduce the impact of outliers on our statistical analysis, we then removed records with a tar spot index above the 99%ile of the distribution (a value of 3.61); this removed a further 18 records. Thus, 1627 of the original 2501 records (about 65%) were retained for analysis. 1327 (82%) of these records were from 2009, the year that the survey was launched, with the remainder in the following years.

The records were submitted from throughout England (Fig. 1), with a greater concentration of records in and around major conurbations such as London, Birmingham, Manchester and Newcastle. Most of the surveys were done by school groups (36%), by individual observers (31%), or with friends and family (21%). About 40% of the surveyed trees were in rural settings and about 60% in urban settings. Over 60% of the surveyed trees were either on a playing field or on a woodland edge, with about 10% being located in each of streets, gardens and farmland. The mean tar spot index ranged from 0.42 to 0.68 in 2010–13, compared to 0.67 in 2009; this suggests that there were no major differences between years, and hence we pooled the data for all years for analysis, matching records for each year to relevant pollution and climate data, as described below.

Modelled $NO_2$ data at a 1 km by 1 km resolution were obtained from the UK Department for Environment Food and Rural Affairs (http://uk-air.defra.gov.uk/data/gis-mapping) for years 2009, 2010, 2011 and 2012; no data were available after 2012, and the records from 2013 were assigned 2012 values. Annual mean concentrations for the relevant record year and the 1 km square containing the recorded trees were then added to the tar spot dataset. Mean monthly temperature, precipitation and relative humidity data at a 5 km scale obtained from the UK Meteorological Office for the years 2009, 2010 and 2011, were similarly added for the relevant record year. Climate data were averaged for the months March to June for each year, since climatic factors are most important during the infection period. The location of each site in terms of a rural/urban gradient was also classified using the classification of the UK Office of National Statistics (ONS, 2011).

Table 1 shows the descriptive statistics for these independent variables. None showed any evidence of non-normal distributions, with the mean and median values being close in each case.

### *2.3. Statistical analysis*

SPSS v 22 was used for all statistical analyses. A significance level of $P < 0.05$ was used in all the ANOVA and regression analysis. Initial investigations were made to determine which, if any, recorded environmental variables influenced leaf width. Differences in mean leaf widths between the three leaf fall categories were compared using one-way ANOVA. For the scale variables ($NO_2$ concentration, temperature, precipitation and relative humidity), the relationship with leaf width was investigated using linear and quadratic regression to assess whether there was evidence of either a linear or nonlinear relationship.

The influence of $NO_2$ concentration in five categories (<10, 10–15, 15–20, 20–25, >25 $\mu g\ m^{-3}$), of fallen leaf category, and of their interaction, on tar spot index was investigated using a two-way ANOVA. Because of the low number of trees in the "none" fallen leaf status, the lower two categories were combined for this analysis. Differences between the $NO_2$ categories were evaluated using Tukey post-hoc tests.

In order to further explain the variation in tar spot index, a multiple regression analysis was conducted of tar spot index on linear and quadratic $NO_2$ concentrations, the two category leaf fall status, temperature, precipitation and relative humidity.

LOWESS (locally weighted scatterplot smoothing), broken stick (Piegorsch and Bailer, 2005) and horizontal broken stick models were used to assess if there was a threshold for the effects of $NO_2$. The broken stick model fits a relationship with two linear sections, hinged at their intersection. The model was fitted by least squares, and an iterative process using $NO_2$ concentration from 5–30 $\mu g\ m^{-3}$ in steps of 1 was used to identify the intersection where $R^2$ was maximised (equivalent to minimising the residual sums of squares). The horizontal broken stick is fitted in the same way except that the initial part of the "stick" is constrained to be horizontal.

## 3. Results

Before analysing the results for the tar spot index, we assessed the effects of environmental variables on leaf width. Regression analysis showed that there was no significant (linear or quadratic) relationship between $NO_2$ and leaf width ($F_{2,1606} = 0.41$; $P = 0.662$), or between temperature and leaf width ($F_{2,1551} = 1.31$; $P = 0.271$). However, there was a significant relationship between precipitation and leaf width ($F_{2,1551} = 3.64$; $P = 0.027$), and between relative humidity and leaf width ($F_{2,1551} = 3.45$; $P = 0.032$), although the % variance explained ($R^2$) in the quadratic models was only 0.5% and 0.4% respectively.

Two-way ANOVA showed that the average tar spot index differed significantly between $NO_2$ categories ($F_{4,1462} = 10.16$, $P < 0.001$), and between the two fallen leaf status categories ($F_{1,1462} = 16.64$, $P < 0.001$), but the interaction between these terms was not significant ($F_{4,1462} = 1.31$, $P = 0.263$). As shown in Fig. 2, average tar spot index was consistently higher where there were more fallen leaves. Furthermore, post-hoc tests showed that the tar spot index was significantly lower at the highest $NO_2$ concentration. However, the $R^2$ for this model (4.8%) was low, a typical feature of large datasets.

The fitted multiple regression model was highly significant ($F_{6,1410} = 26.98$; $P < 0.001$), as were each of the individual model terms (Table 2). Inclusion of temperature, precipitation and rainfall, with a quadratic relationship to $NO_2$ concentration, increased the percentage variance explained to 10.3%. Colinearity between climate variables and $NO_2$ concentration was not a problem (VIF<3). The tar spot index was increased at sites with high rainfall, low temperature, and low humidity, but the index still showed a positive relationship with the fallen leaf index and a significant relationship with $NO_2$ concentration.

The relationship between tar spot index and $NO_2$ concentration shown in Fig 2 suggests that there is a threshold for $NO_2$ effects in the presence of fallen leaves around 20 $\mu g\ m^{-3}$. This was investigated in more detail, initially using a LOWESS line (Fig. 3). This confirmed that there might be a threshold at about 20 $\mu g\ m^{-3}$ above which $NO_2$ concentration had a stronger influence on tar spot index. Subsequently a broken stick model was fitted (Fig. 3), which had an intersection at 19 $\mu g\ m^{-3}$, with a slope before the intersection of $0.008 \pm 0.004$ and a slope above the intersection of $-0.042 \pm 0.009$. A horizontal broken stick model was also fitted (Fig 3). This had an intersection at 20 $\mu g\ m^{-3}$; the slope after the intersection in this case was $-0.032 \pm 0.006$. Both models therefore suggest a threshold in the region of 20 $\mu g\ m^{-3}$ with a sharp decline in tar spot index above this concentration. Both models had low $R^2$ values (1.7% for the broken stick; 1.5% for the horizontal broken stick). As noted above, this is typical of such large datasets, and both models were statistically significant. Furthermore, both broken stick models were better fits than either simple linear ($R^2 = 0.5\%$) or quadratic ($R^2 = 1.4\%$) regression models.

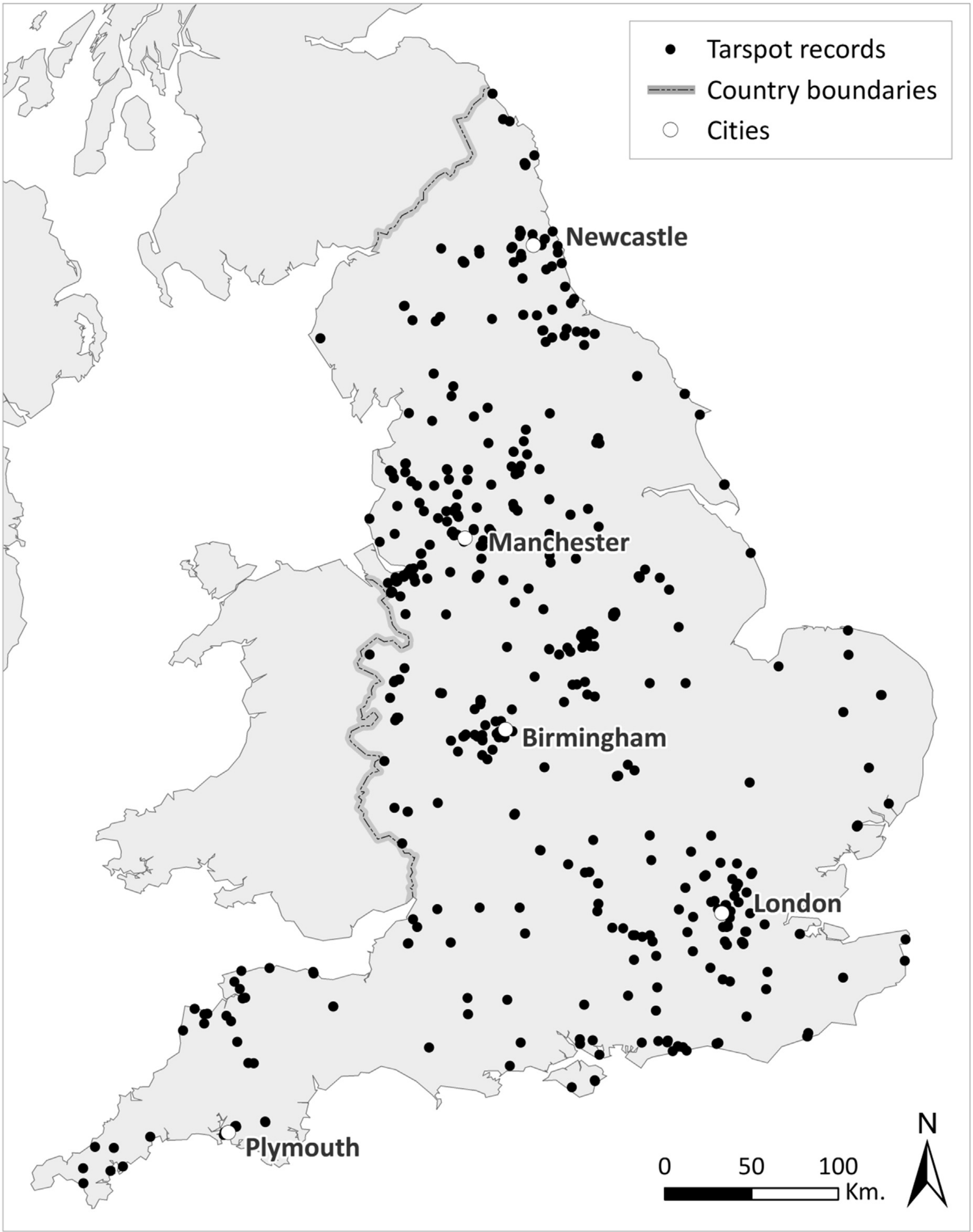


**Fig. 1.** Map showing location of records for England that were used in the analysis.

## 4. Discussion

The involvement of hundreds of citizen scientists in the OPAL air survey provided a volume and spatial coverage of data that was much greater than that of conventional scientific surveys of air pollution effects (Seed et al., 2013; Tregidgo et al., 2013), and this allowed us to make a more rigorous assessment of the effects of different factors influencing leaf symptom severity at a large spatial scale. Although we did not actively plan the spatial distribution of observations, the records were spread across the country, and showed a good balance between rural and urban sites. This may be partly explained by the participation model used in OPAL, with community scientists based in nine regions of the country actively working with, and training, individuals and groups to deliver the

**Table 1**
Descriptive statistics for independent environmental variables used in this study, including air temperature (°C), precipitation (mm) and relative humidity (%) (all March to June monthly mean values) and annual mean $NO_2$ concentration ($\mu g\ m^{-3}$).

| Variable | Mean | Median | Standard deviation | Minimum | Maximum |
|---|---|---|---|---|---|
| Temperature | 10.8 | 10.9 | 0.76 | 8 | 13 |
| Precipitation | 47.2 | 43.3 | 14.6 | 21 | 98 |
| Humidity | 77.7 | 77.7 | 2.87 | 69 | 86 |
| $NO_2$ | 15.7 | 15.5 | 6.63 | 4.4 | 39.3 |

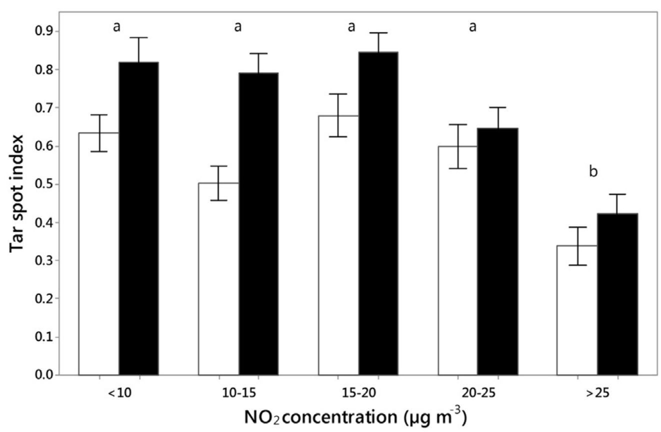


**Fig. 2.** Mean ± SE tar spot index for each of five $NO_2$ concentration ($\mu g\ m^{-3}$) categories and two fallen leaf status categories. The unshaded bars represent values with no or few fallen leaves, and the shaded bars represent values for many fallen leaves. $NO_2$ concentrations with a different letter heading the column have significantly different mean tar spot indices, based on Tukey post-hoc tests.

**Table 2**
Summary of multiple regression model of best fit to explain variation in tar spot index. The table shows the value (and standard error) of the constant, and the individual regression coefficients, and the t value and statistical significance of each term in the model.

| Variable | Regression coefficient (SE) | t | P |
|---|---|---|---|
| Constant | 3.812 (0.853) | 4.47 | <0.001 |
| Temperature | −0.191 (0.028) | −6.88 | <0.001 |
| Precipitation | 0.007 (0.001) | 5.50 | <0.001 |
| Relative humidity | −0.024 (0.009) | −2.55 | 0.011 |
| Fallen leaves | 0.120 (0.035) | 3.40 | 0.001 |
| $NO_2$ linear | 0.042 (0.012) | 3.37 | 0.001 |
| $NO_2$ quadratic | −0.001 (0.000) | −4.05 | <0.001 |

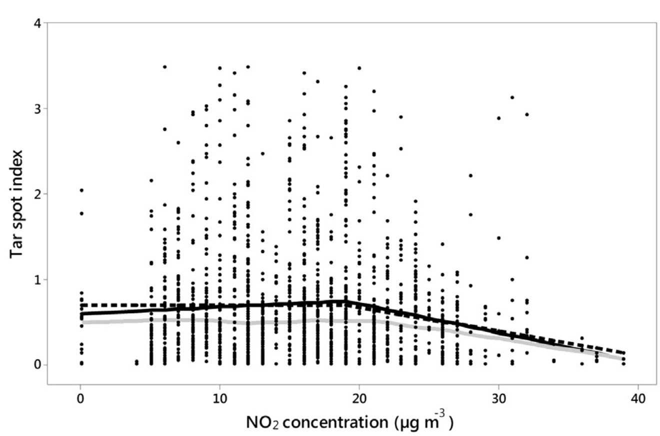


**Fig. 3.** Relationship between $NO_2$ concentration ($\mu g\ m^{-3}$) and tar spot index. The LOWESS line is shown in grey, the broken stick model in black and the horizontal broken stick model as a black dashed line.

survey.

We did not directly assess the accuracy of the records provided by our citizen scientists, although we did remove records that had missing information, were from the wrong months, or were judged to be biologically implausible; this resulted in about 35% of the original data being rejected, with 65% retained for analysis. Hence the majority of the citizen scientists provided data that was appropriate for analysis. We were not able to directly determine the accuracy of the records which were used to derive the tar spot index, but previous OPAL surveys that have overlain citizen science records with national soil databases (Bone et al., 2012) or with models of aerial contrails (Fowler et al., 2013) suggest that the majority of the records are reliable or credible. Previous OPAL surveys have identified mis-identification of species as a significant factor affecting data quality for lichens (Seed et al., 2013) and urban invertebrates (Bates et al., 2015), especially for school children and those with little previous identification experience. However, the tar spot survey required no challenging species identification (only tree species, which is relatively simple) and the black spots are very easy to identify; hence the only likely error sources are in counting spot numbers or measuring leaf width.

The large degree of scatter in the data may partly reflect errors made by individual participants in the recording process. However, large scatter is also typical of field surveys that do not rely on citizen scientists, most likely in the case of our survey due to the effects of environmental factors that were not recorded. A further important cause of scatter may be that climate and pollution variables were average values at 5 km and 1 km scales respectively, and may not accurately reflect conditions in the immediate vicinity of the surveyed trees. Our fitted model with fallen leaves and $NO_2$ concentration only explained 5% of the variation in tar spot index; addition of the climatic variables temperature, rainfall, and relative humidity increased this to about 10%. Nevertheless, the effects of the environmental variables were highly significant. This is a common finding in large datasets with a limited number of explanatory variables; for example Smart et al. (2004) found significant relationships between plant species composition in different habitats and ammonium deposition across Britain, but these only explained 5–10% of the spatial variation.

There is strong evidence from field surveys carried out in the 1970s that $SO_2$ had a significant effect in reducing the incidence of tar spot, alongside that of other diseases such as black spot of roses. Our analysis did not consider $SO_2$ because annual mean $SO_2$ concentrations are now below 10 $\mu g\ m^{-3}$ throughout England, apart from a few isolated locations affected by local industrial sources (http://uk-air.defra.gov.uk/data/gis-mapping). This concentration is an order of magnitude lower than the thresholds for effects on tar spot reported by Vick and Bevan (1976) and hence we can exclude the possibility that $SO_2$ any longer has a significant effect on tar spot incidence. This is supported by field observations of re-invasion of tar spot when $SO_2$ concentrations fell rapidly in UK coalfield areas (e.g. South Yorkshire, Jarraud, 2000; south-east Northumberland, Davison pers.comm.).

Our finding that the extent to which fallen leaves are left undisturbed does influence the tar spot index supports the findings of Leith and Fowler (1988). The mechanisms for this effect are almost certainly linked to the need for a local source of inoculum for the new leaves each growing season; Leith and Fowler (1988) were able to demonstrate that horizontal transfer of ascospores is limited, so that most trees are likely to be infected by litter produced from that or neighbouring trees. However, their conclusion that clearing of leaves by wind movement or street cleaning was the main factor restricting tar spot growth in urban areas is not supported by our results, which demonstrate a significant effect of $NO_2$ concentration that was independent of any effect of fallen leaves.

In contrast to ozone and $SO_2$, there have been relatively few studies of the effects of $NO_2$ on leaf surface fungi. Furthermore, the dominant source of $NO_2$ in the UK is now road transport, and spatial variation in $NO_2$ may be highly correlated with other pollutants related to vehicle emissions, including nitric oxide (NO), particulate matter and VOCs. Therefore we cannot exclude the possibility that the causal agent was a pollutant other than $NO_2$. While direct effects of $NO_2$ on the *Rhytisma acerinum* fungus may be one relevant mechanism, changes in leaf surface characteristics may also be a significant factor. For example, Bell et al. (2011a) demonstrated a significant decline in leaf surface contact angle of two herbaceous species with proximity to a major urban road in London, while Novakova and Neustupa (2015) found significant changes in the species composition of biofilms on *Taxus baccata* needles with pollution levels in Prague, with the presence of microalgae being a strong positive indicator of higher $NO_2$ concentrations.

Our survey data suggest that there is a possible threshold for effects of $NO_2$ at around 20 μg $m^{-3}$ Jarraud (2000) compared tar spot incidence along a gradient of six sites from central London to the surrounding rural areas and found a clear break point between sites with $NO_2$ concentrations below 20 μg $m^{-3}$, at which infection was relatively high, and those with higher concentrations, at which relatively few tar spot symptoms were found. Similar results were found when *Acer pseudoplanatus* saplings were exposed alongside standard inocula at the sites, suggesting that this is an $NO_2$ threshold for effects on the infection process (Bell et al., 2011b). A threshold effect for $NO_2$ effects in London was also reported by Davies et al. (2007) in a study of lichen diversity, although in this case the estimated concentration was 40 μg $m^{-3}$. In the case of effects on lichens and biofilm species, the presence of organisms that are known indicators of high nitrogen can support the inference of a causal effect of $NO_2$. However, this is not the case for tar spot, and we cannot exclude the possibility that other pollutants, or other environmental factors, that are positively or negatively correlated with $NO_2$ concentrations, were responsible for the observed effects. We also have few observations at concentrations above 30 μg $m^{-3}$ on which to base any assessment of a threshold for effects. Experimental studies, using a wide range of $NO_2$ concentrations, are needed to confirm that $NO_2$ is toxic to tar spot and to confirm the threshold for effects.

Our multiple regression analysis indicated that the tar spot index was greater at cooler sites, and sites with greater precipitation, as expected from previous studies of conditions which favour fungal infection. However, the tar spot index was lower at sites with high humidity; this most likely reflects the significant correlation of this variable with both temperature and precipitation, both of which had a larger effect on the tar spot index. We used climate data for the period March–June, on the assumption that the impacts of temperature, rainfall and humidity would be greatest during the period of infection and tar spot growth, although we cannot exclude the possibility that there were effects at other times of year (e.g. on spore survival over winter). Climatic extremes may also be important, but maximum and minimum values of every climatic variable are very closely correlated with the mean values that were used in our analysis. There were significant effects of precipitation and relative humidity on leaf width, but the effects of these variables on tar spot index through changes in leaf width was small compared to that through changes in tar spot numbers.

The accuracy of our assessment of the effects of climatic variables and $NO_2$ concentration is constrained by the use of spatial average values, in contrast to leaf fall, for which the data were site-specific. However, collection of more local climate and pollution data was not feasible in a large-scale citizen science survey; our observers did not have the relevant expertise or equipment and could reliably only make simple observations of visible tar spot symptoms. Since our observations were scattered over the country, rather than within an individual city, there is no reason to expect that the use of spatial averages would introduce a systematic bias; likewise, the correlation between microclimate and pollution concentrations that might be expected within a city is less important at a national scale, meaning that the VIF values were low enough to allow us to identify the independent effects of climatic variables and those of $NO_2$.

In conclusion, our citizen science survey provided a national dataset on tar spot symptom severity, air pollution and other environmental drivers, which was much greater in both geographical scale and site numbers than all of the relevant published scientific studies. While the methods were simplified, the quality of individual records is uncertain, and we had no control of the spatial distribution of records, the results have allowed us to test two competing hypotheses about major drivers of symptom severity. We were also able to demonstrate independent effects of leaf fall, $NO_2$ concentration and climatic factors and for the first time to identify a clear threshold $NO_2$ concentration for effects. This experience strongly suggests that greater use of well-designed citizen science programmes could make a significant future contribution to our understanding of the impacts of air pollution in the field.

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