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Trinity College Botanic Garden long-term monitoring program V.2

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We use this protocol and it's working

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Abstract

Botanic gardens hold large, documented, and accessible collections of living plants. These represent unique subsets of taxa from different biogeographical regions growing under common environmental conditions, connecting people to global plant research and conservation efforts while offering a place beneficial for human health and wellbeing. Despite Botanic Gardens being an ideal setting for climate change research, their potential for comparative, long-term studies and outreach in the field is still underutilised. As part of its ten year strategy, Trinity College Botanic Garden (TCBG) aims to tap this potential and establish a programme for long-term (>30 years) monitoring of key physiological performances in its living woody plant collection. The programme will also assess particulate pollution (PM10 and PM2.5) interception by the same trees, pairing climate change and urban green research. Importantly, the project will include the design of a transferable protocol, produce vouchered herbarium specimens as a future historical archive and as a pedagogical tool, and support the garden outreach strategy, so as to nurture its link with both Trinity College Dublin and local communities, ensuring the garden's legacy into the future.

Guidelines

These are the guidelines for the monitoring currently in place in TCBG. The trees that are being monitored to date are listed [here](#). The code used for data manipulation and herbarium label making in previous rounds of monitoring can be found on GitHub (<https://github.com/midoriyajima/The-Witness-Tree-Project/tree/main>).

Fieldwork has to be performed at the end of the growing season, which in Ireland corresponds to June, to not later than mid-July. Plants should not be water stressed, e.g. measurements should not be performed after prolonged dry spells.

The data management plan can be found at [DMPonline](#) (ID 103451)

Data generated in previous years can be stored on Dryad (<https://doi.org/10.5061/dryad.b8gtth7h7>)

Materials

Key: Number of items - Where they are stored - What they are used for

1. N. 21 ribbons - TCBG - all fieldwork
2. N. 1 GARMIN eTrex10 - TCD Paleobotany lab - all fieldwork
3. N. 1 SC-1 Porometer - TCD Paleobotany lab - porometry
4. N. 1 PhotosynQ - TCD Paleobotany lab - chlorophyll content and environmental data
5. N. 1 Pocket Pea - TCD Paleobotany lab - fluorometry
6. N. 126 envelopes for preserving leaves - TCD palaeoecology lab - wue, particular matter analysis
7. N. 1 mortar and pestle - TCD palaeoecology lab - wue
8. Ethanol - TCD palaeoecology lab - wue
9. Deionised Water - TCD palaeoecology lab - wue
10. Sieve - TCD palaeoecology lab - wue
11. Aluminium foil - TCD palaeoecology lab - wue
12. N. 63 eppendorf tubes - TCD palaeoecology lab - wue
13. N. 63 tin foil capsules - TCD palaeoecology lab - wue
14. N. 1 Spatula - TCD palaeoecology lab - wue
15. N. 3 tweezers - TCD palaeoecology lab - wue, particular matter analysis
16. N. 1 96-well tray - TCD palaeoecology lab - wue
17. N. 100 (depending on dimension) lint-free tissues - TCBG - particular matter analysis
18. N.2 plant presses - TCD herbarium - particular matter analysis, herbarium specimens
19. Newspapers - TCD herbarium - particular matter analysis, herbarium specimens
20. N.1 single edged blade - TCD palaeoecology lab - particular matter analysis
21. N. 63 SEM stubs - CMA/ iCRAG lab - particular matter analysis
22. N. 63 conductive tabs - CMA/ iCRAG lab - particular matter analysis
23. Carbon paint (pure grahene) - CMA/ iCRAG lab - particular matter analysis
24. N. 21 leaf tags - TCD herbarium - herbarium specimens
25. Herbarium materials (sheets, stitching material, glue) - TCD herbarium - herbarium specimens

Troubleshooting

Plant physiology

1 When

June, not later than mid-July (end of the growing season).

Make sure plants are not water stressed, e.g. do not perform measurements after prolonged dry spells.

1.1 Leaf selection

1.1.1 Select **3 leaves/ leaflets/ phyllodes** (from here on all referred to as leaves) **per tree**

Note

Each leaf has to be:

- Fully expanded (the developmental stage at which many leaf traits are relatively stable)
- Sun-exposed, or exposed to sun flecks
- Healthy, pest- and pathogen-free
- Outermost reachable leaves for tall trees
- Dry and preferably flat - porometer head has to be horizontal

1.1.2 Per each leaf

- **Tag** leaf with a ribbon¹ reporting **a code** that will work as leaf ID (e.g. the first leaf measured on *Arbutus unedo* will be AU01)
- **Make note** of leaf orientation (the N/S/E/W direction the leaf is facing)
- **Make note** of GPS² position of the tree (for any tree that have not been measured in previous years)

1.2 Porometry

Using porometer³, to measure plants' stomatal conductance (gs)

1.2.1 Take 1 **porometry reading** x each leaf x each tree

- Once per day between 08:30 h and 14:00h
- Over 3-5 consecutive days. To account for the natural day-to-day variability in gs for each species under ambient conditions, follow a different order of trees and leaves measured every day.

Note

Equipment-related:

- Calibrate every day or if temp changes of ca 15 °C
- Measure at the prevailing microclimate condition of that day
- Measure in automatic mode - measures continuously and derive best estimate for leaf

Leaves-related:

- Measure at the interveinal areolae at mid-lamina of the abaxial surface. In the case of compound leaves, measure the terminal leaflet; for larger leaves clamp the sensor as far onto the leaf as possible, taking care to avoid damage to the leaf margin
- Measure leaves at the same position in the crown (e.g. base level, arm reach), to normalise for gs changes with tree height
- Measure always at the same position (e.g. right blade of leaf that face me)

During the measurements:

- Minimise factors interfering with the readings (look away or wear mask to avoid changing CO2 conditions locally, move the leaf as little as possible)
- Name readings with each leaf ID

1.2.2 Download data from the porometer at the end of each day

- Download software from website (see Resources below)
- Follow instructions as reported in the porometer's manual

1.2.3 Store data in double copy (e.g. computer/ hard drive and Google Drive)

Note

Resources

Porometer manuals and software can be found at:

<https://www.metergroup.com/en/meter-environment/products/sc-1-leaf-porometer/sc-1-leaf-porometer-support>

1.3 Chlorophyll content and environmental data

Using PhotosynQ⁴, to measure chlorophyll content and environmental variables at the leaf level (PAR, temperature etc.)

1.3.1 Download the mobile app (needs and Android phone), set a project to record observations

1.3.2 On the same tagged leaves used for porometry (possibly clamping the same portion), soon after or right before porometry, take 1 reading x each leaf x each tree. Annotate leaf ID on the app after every measurement.

1.3.3 Data will be uploaded automatically to the project online, but make sure to download them from the website at the end of every day.

1.3.4 Store data in double copy (e.g. computer/ hard drive and Google Drive)

Note

Resources

Instructions, mobile, and web app:

<https://www.photosynq.com/software>

1.4 Fluorometry

Using Pocket pea⁵, to measure chlorophyll fluorescence

1.4.1 At the end of the day, clip 1 leaf x 15 leaves x tree.

- There won't be enough leaf clips for measuring all the trees, so it will be necessary to repeat this every day until all the trees will be assessed. This will have to be done only once per tree and not repeated over the 3-5 days, as chlorophyll fluorescence won't change much over that period

Note

- Choose sun exposed leaves or leaves exposed to sun flecks
- Choose healthy leaves and pest/pathogen free if possible
- Do not clip leaf veins
- Check from the clip the appearance of leaf tissue before closing slides

1.4.2 Close each slide on leaf clips to dark adapt leaves, and leave for the night

1.4.3 Take measurements as the first thing in the following morning

- Place pocket pea on each clip covering the opening, slide clip open, take measurement, take clip off

1.4.4 Download data

- Download PEA+ Software from website
- Connect Pocket pea to software through USB port
- Download all data
- Erase data from instrument

1.4.5 Store data in double copy (e.g. computer/ hard drive and Google Drive)

Note

Resources

More information

<https://www.hansatech-instruments.com/product/pocket-pea/>

Software download

<https://www.hansatech-instruments.com/download-category/software/>

1.5 Water use efficiency (WUE)

Obtained by analysing ^{13}C isotopes ratio from leaves samples

1.5.1 On **the last day of fieldwork**, take the same tagged leaves used for porometry and sort them in separate envelopes⁶, pencil the leaf ID on the envelopes

1.5.2 **Oven dry** at 50-60 °C for 2 days in TCD plant atmosphere lab

1.5.3 In the plant atmosphere lab, for each sample take a fragment of the dried leaf and grind it with mortar and pestle⁷ into a fine powder

Note

- Use gloves throughout the whole process to avoid contamination of samples
- Make sure to clean mortar and pestel with Ethanol⁸ and Deionised Water⁹ after every sample has been ground to avoid contamination of the following sample

1.5.4 Sieve¹⁰ powder on an aluminium¹¹ foil

1.5.5 Transfer powder into an eppendorf¹² and label with leaf ID

1.5.6 In the anatomy lab, transfer ca. 3 mg of powder into tin foil capsules¹³ using a spatula¹⁴ and fold them using two tweezers¹⁵ into small quadrats of <5 mm length

Note

- To weight samples make sure to weight the empty tin capsule, holding it with a pair of tweezers, and then transfer powder and weight again, until the net weight will be around 3 mg
- Make sure to clean spatula and tweezers after every sample with ethanol

1.5.7 Sort samples in a 96 well tray¹⁶, keeping track of the position of each sample and correspondent leaf ID

1.5.8 Place an order to the Stable Isotope Facility (SIF) in UC Davis for ^{13}C analysis, pack the tray following the guidelines on their website (stating Prof. Jennifer McElwain as PI of the lab) and send it. Turnaround times are expected to be of ca. 2-3 months.

1.5.9 Once data come back, derive WUE from the isotope ratio using formula from Soh et al.2019

1.5.10 Store data in double copy (e.g. computer/ hard drive and Google Drive)

Note

Resources

More information for preparing samples and placing order with SIF

<https://stableisotopefacility.ucdavis.edu/sample-preparation-carbon-and-nitrogen-solids>

<https://stableisotopefacility.ucdavis.edu/order-placement>

Formula from Soh et al.2019

<https://www.science.org/doi/10.1126/sciadv.aax7906>

1.6 Weather data

Take weather data from the garden MET station

- Download/ obtain Campbell scientific LoggerNet software
- Download readings from the 30 days before the monitoring (monitoring days included) from the weather station, using a USB to RS232 cable

Note

Resources

Software information and manual

<https://www.campbellsci.com/loggernet>

1.7 Data management

- **Clean data** (variable names in lowercase and no spaces, keep only relevant info), for porometry, PhotosynQ, WUE, fluorometry, and MET dataset. See the script "R/Monitoring_data.R" in the **GitHub repo** as a reference (link in **Resources** below). To push changes, please branch the main repo before, and once all changes are made create a pull request to merge it back.
- Convert final **dataset in .csv format**, as the preferred format for reproducibility (not necessary if the script "R/Monitoring_data.R" is followed as all datasets are automatically saved in that format).



Note

Resources

GitHub repo <https://github.com/midoriyajima/The-Witness-Tree-Project>

Info on branching and creating pull requests in GitHub <https://docs.github.com/en/pull-requests/collaborating-with-pull-requests/proposing-changes-to-your-work-with-pull-requests/about-branches>

Particulate Matter analysis

2 When

Collection must be after a period of 5 to 10 days of dry weather and low wind, to avoid particulates to be washed away from the leaves. It has to be close in time to the plant physiology fieldwork.

2.1 Leaf selection

- **3 leaves x tree**, choosing leaves **close to the ones used for the physiological assessment** (same branch, similar dimension and orientation if possible)

Note

Each leaf has to be

- Healthy, pest- and pathogen-free
- Collected at basal exterior canopy

2.2 Leaf collection and dehydration

2.2.1 Pick leaves and **tag** them with a leaf ID (can be the same used for the leaves nearby used for the physiology work) + progressive numbering. The latter will be the **stub ID** that will be used in Scanning Electron Microscopy (SEM) later

2.2.2 Wrap leaves in lint-free tissue¹⁷ to avoid contamination from paper fibres or other sources while pressing

2.2.3 Dehydrate leaves in plant press¹⁸ for approx. 2 weeks, changing newspapers¹⁹ regularly. Leaves need to be pressed and not oven dried so that can be imaged correctly under the SEM later

2.2.4 Sort dried leaves in separate envelopes⁶ reporting leaf ID and sample ID, keeping them wrapped in lint free tissue

2.3 Samples preparation in the Centre for Microscopy and Analysis lab

2.3.1 Cut 1 sample of 0.5×0.5 cm ca from each leaf with single edged blade²⁰

Note

Each sample has to be

- Cut from the central portion of the leaf, close to midvein, halfway from the bottom to the top of the leaves, right blade (positioning the leaf tip downwards) as in the section where porometry was done on the other leaves

2.3.2 Prepare SEM stubs²¹ with conductive tabs²²

2.3.3 Using tweezers¹⁵, mount 1 sample x stub and press firmly with lint-free tissue

2.3.4 Paint the edges of the sample with carbon paint²⁴ (pure graphene) to prevent curling while in the SEM

2.3.5 Mark the bottom of the stub with the unique stub ID that was given during collection

Note

Tip

Keep track of leaf ID, corresponding s ID, and any note on samples throughout the analyses (e.g. sample charging under the SEM, sample damaged)

2.3.6 Leave samples mounted on the stubs to dry out for 1-2 days

2.4 Initialise TESCAN MIRA3 TIGER SEM

2.4.1 Initialise SEM by hitting STANDBY button on screen to get it out of standby mode

2.4.2 Hit VENT button to vent the chamber and be able to open it

2.4.3 When the carousel in the stage control panel (bottom left screen) becomes red, click on central sample in carousel to make it advance

2.4.4 Open chamber, pull carousel out making sure it doesn't hit the central lens, and insert the stubs in the carousel noting down the stub ID per each position in the carousel. Screw stubs in carousel lightly, then put carousel back in and close chamber

2.4.5 Hit PUMP button to pump chamber again

2.4.6 Type 0 in stage control panel (bottom left screen) at the position X to move stage back into place. Hover mouse over stop button to interrupt movement, just in case

2.4.7 Type 15 mm in stage control panel (bottom left screen) at the position WD & Z to move stage at the right distance. Hover mouse over stop button to interrupt movement, just in case

2.4.8 Hit BEAM ON to turn the beam on, scroll mouse wheel to control screen "updating" speed

2.4.9 Right click on image on bottom left screen and select "auto bright contrast" to adjust the contrast of the image

2.4.10 Find an object to visualize and and hit Adjustment option in bottom right screen, then align instrument from the top down hitting Auto gun, Auto column centring, Manual column centring. The last one will open a window, where to click on WOB, increase magnification if necessary by scrolling the wheel on the console, and adjust the ball on the console by moving it left/ right until image is not moving anymore

2.4.11 Hit Magnet button on bottom right screen to degauss. This will prevent magnetism in the chamber that could affect image quality and measurements

2.4.12 Set microscope settings, On the Aztec software (top right screen), make a new project and switch on EDS Sensors from Aztec interface (bottom right icons) > Position > In

Note

On the TIGER set

- HV 15
- speed 3
- WD 15
- Beam intensity 10

On the AZtec software, set Energy Dispersive X-Ray Spectroscopy (EDS) with

- Resolution 512
- Acquisition time fixed duration, frame count 1
- Number of channels 1024
- Process time 2
- Pixel dwell time 500 microsec

2.5 Map elements at SEM

2.5.1 Turn IR light off right-clicking on bottom right image

2.5.2 Selecting SE in the Channel A entry from SEM detectors & Mixer panel if any area is charging, and analyse only areas that are not

2.5.3 Put back BSE option in Channel A entry

2.5.4 For each sample, pick 3 random areas, increase magnification up to 800 X

**Note****Tips**

- Move the image with arrows in stage control at Z position
- Degauss regularly
- If image needed from main console, hit Acquire image option on console, set acquisition options, as Acquisition time: 32 sec, Window 1024x768. Note image number down and after saving them on own drive, move them in Archive folder, to leave image folder on computer empty

2.5.5 Map section in Aztec, make new site for every area analysed in the "data tree" window, and label it meaningfully e.g. 1st area analysed of leaf CS01 will be CS01_01

2.5.6 In Scan image section, hit Play button to scan the image

2.5.7 In Acquire map data, hit Play button to map elements

Note

- When new samples need to go in, go at low mag, hit VENT and do same things as in Insert samples
- Keep spreadsheet or notes of sample ID, corresponding ID on stub, subsample ID, and note down any other note e.g. sample charging, impossible to analyse

2.5.8 Turn SEM off once finished by going at low magnification, hit In Acquire map data, hit Play button to map elements

2.6 Feature analysis

2.6.1 Download project from SEM on hard drive

2.6.1 Upload project on offline computer and start it

2.6.2 Go on feature analysis option

2.6.3 On the Detect feature tab, right click on image (only electron image, not the composite), export as tiff, name with subsample ID

2.6.4 Threshold features by appearance on Detection refinement window.

Adjust the selection window to select relevant part of the spectrum corresponding to particles by appearance (PM will be distinguishable from the background, can be selected highlighting lighter part of the spectrum for heavy metals, darker for carbon-based particulates).

Adjust filter settings to better select PM (e.g. use erosion filter if feature is bigger than actual PM when observed in the background). Features should be detected automatically, if not hit detect features

2.6.5 On acquire site window

On Data tab, check morphology, chemistry, area, field, class boxes

Extract to get chemical data. Make sure to click on the whole site (not on electron image) or it will not get data

Select artefacts (features that were selected by mistake) from the image using the option "Select feature at the point of the image", and remove it using "Reject function bottom of the Data/ Summary/Single section

2.6.6 On acquire site window file as the subsample and delete summary rows at the bottom (mean, etc.). Repeat for all the subsamples.

2.7 Data management

- **Merge datasets** and **clean data** (variable names in lowercase and no spaces, keep only relevant info). See the script "R/Monitoring_data.R" in the **GitHub repo** as a reference (link in **Resources** below). To push changes, please branch the main repo before, and once all changes are made create a pull request to merge it back to the main branch.
- Convert final **dataset in .csv format**, as the preferred format for reproducibility (not necessary if the script "R/Monitoring_data.R" is followed as all datasets are automatically saved in that format).

Note

Resources

GitHub repo <https://github.com/midoriyajima/The-Witness-Tree-Project>

Info on branching and creating pull requests in GitHub <https://docs.github.com/en/pull-requests/collaborating-with-pull-requests/proposing-changes-to-your-work-with-pull-requests/about-branches>

Herbarium sheets

3 When

Collection must happen soon after the last day of plant physiology fieldwork. It must be in dry weather to avoid specimens rotting or developing moulds.

3.1 Selection of the specimen

3.1.1 Select and pick **1 specimen x tree** for the actual herbarium sheet + **2/3 extra leaves** (or leaflets) to preserve for any future analysis. Tag specimens with **leaves**



tags²⁶ reporting species (to avoid mislabelling later)

Note

- Each specimen has to include any flower or fruit found, if possible. Specimens can be branches or separate leaflets in case of trees that whose growth could be damaged by annual cuttings (e.g. palm tree)
- Close to the leaves used for porometry/ elemental analysis and PM analysis
- Healthy looking

3.1.2 Label each specimen annotating species name

3.1.3 Separately, make note of

- **Species name**
- **Specimen ID**, as "TCBG sample-number" e.g. "TBCG1". Each specimen ID must be unique, so latest specimens have to continue the numbering of the herbarium sheets made in previous years. See resources in **Section 3.3** for a link to labels of specimens of previous years
- **Date**
- **Collectors**
- **Coordinates** (can be taken from previous specimens)
- **Location** in the garden (e.g. walled garden, west arboretum etc. can be taken from previous specimens)
- **Notes**, including branch height when on the tree, ecological notes

3.1.4 Place specimen in plastic bag for transport (ideally one bag per specimen)

3.2 Preparation of specimens

3.2.1 Dehydrate plants in plant press¹⁸ within 12 hours from collection. Store the plant press in a dry place for approx. 2 weeks, changing newspapers regularly

Note

For each specimen

- Arrange it as flat as possible
- Arrange any flower or fruit in a way that they do not overlap
- Arrange so that specimen shows both sides of the leaves e.g. turning one leaf to show the back
- If the specimen is too big for the press, bend it

3.2.2 Once ready, drop the press with all the specimen in the Herbarium freezer inside the Botany Department for at least 72 hours, to kill any residual mold.

3.3 Creating herbarium labels

3.3.1 See the script "R/ Herbarium label" in the **GitHub repo** as a reference (link in **Resources** below). To push changes, please branch the main repo before, and once all changes are made create a pull request to merge it back. **Add own spreadsheet** following the structure of files published in its "Data/ Herbarium data" section. Fill it with information noted in **Section 3.1.3**.

3.3.2 Manually move the generated .rtf file from the main repo to the dedicated folder in "Outputs/Herbarium labels"

Note

Resources

GitHub repo including herbarium spreadsheet and code for creating labels

<https://github.com/midoriyajima/TCBG/tree/main>

3.4 Mounting of herbarium specimens

3.4.1 Mount specimens on herbarium sheets, making sure to

- Stitch specimens instead of using glue. Use glue only if necessary (e.g. specimen is too heavy, there are too many leaves) so that each specimen can be removed and used for future analyses
- Storing the extra leaves in a separate paper bag attached to the sheet
- Printing and attaching herbarium labels reporting the information noted on the field

3.4.2 Lay herbarium sheets away in Trinity Herbarium

- Check for Herbarium ID numbers for the species in the Herbarium book
- Seek out the respective cabinet with the ID number
- Lay in folders for the respective species, section "Cultivated"



Expected result





Online data publishing

4 When

Once all data and metadata have been obtained and cleaned

4.1 Upload datasets to Dryad

4.1.1 Prepare a README file (see details in **Resources** below)

4.1.2 Follow steps reported in Dryad's website to create **new version of dataset** (see details in **Resources** below). This requires login details of first author, please reach out at yajimamidorimichela@gmail.com

Note

Resources

README file https://datadryad.org/stash/best_practices#describe-your-dataset-in-a-readme-file, <https://datadryad.org/docs/README.md>

Dataset to version <https://datadryad.org/stash/dataset/doi:10.5061/dryad.b8gtht7h7>

Data visualization for the Witness Tree Project webpage

5 When

Once all data and metadata have been obtained and cleaned

5.1 Create visualization

5.1.1 See the script "R/Viz for website" in the **GitHub repo** as a reference (link in **Resources** below). To push changes, please branch the main repo before, and once all changes are made create a pull request to merge it back. Please note that the script needs cleaning, for any issue reach out to Midori Yajima (yajimamidorimichela@gmail.com)

5.1.2 Send materials to designated garden staff to be uploaded in Trinity College Botanic Garden's website

**Note****Resources**GitHub repo <https://github.com/midoriyajima/The-Witness-Tree-Project>

Updating monitoring records

6 **When**

At the end of field/ lab work

6.1 **Update records for the monitoring**

6.1.1 Branch the **GitHub repo** (link in Resources below) and update the file in "Data/Tree_list". In particular, update the "Monitoring record" tab with details on year, month, features monitored, staff involved.

6.1.2 Create a pull request to merge changes made to the main repo

6.2 **Update protocol** (optional)

If any changes were implemented during the field or lab work, **update this protocol** either by creating a fork or versioning the original one. The latter will require login details from one of the authors (reach out to yajimamidorimichela@gmail.com)

Note**Resources**Updating protocols.io <https://www.protocols.io/help/contribute>



Protocol references

The protocol for measuring stomatal conductance, and water use efficiency in the field has been adapted from

Murray M, Soh WK, Yiotis C, Batke S, Parnell AC, Spicer RA, Lawson T, Caballero R, Wright IJ, Purcell C and McElwain JC (2019). Convergence in Maximum Stomatal Conductance of C₃ Woody Angiosperms in Natural Ecosystems Across Bioclimatic Zones. *Front. Plant Sci.*10:558. doi: 10.3389/fpls.2019.00558

Soh WK, Yiotis C, Murray M, Parnell A, Wright IJ, Spicer RA, Lawson T, Caballero R, McElwain JC (2019). Rising CO₂ drives divergence in water use efficiency of evergreen and deciduous plants. *Sci. Adv.* 5, eaax7906

The protocol for quantifying PM deposition on leaves has been adapted from

Baldacchini C, Castanheiro A, Maghakyan N, Sgrigna G, Verhelst J, Alonso R, Amorim JH., Bellan P, Đunisijević BD, et al. (2017) How does the amount and composition of PM deposited on *Platanus acerifolia* leaves change across different cities in Europe? *Environmental science and technology / American Chemical Society* - ISSN 0013-936X - 51:3, p. 1147-1156