Trinity College Botanic Garden long-term monitoring program

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Protocol status: In development
We are still developing and optimizing this protocol

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DISCLAIMER

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 C_3Woody 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 interception 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

Keywords: Long-term, monitoring, plant, ecology, stomatal conductance, pollution, PM10, PM2.5, trees, climate change, botanic garden

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 (link to come).

The **stomatal conductance (gs), leaf area index (LAI), elemental analysis/ water use efficiency (WUE), particulate matter analysis, herbarium specimens making** have to be performed at the end of the growing season, which in Ireland corresponds from July, to not later than mid-August.

The data management plan can be found at DMPonline (ID 103451)
Data generated in previous years can be found on Dryad (link to come)

MATERIALS

Key: Number of items - where they are kept [What they are used for]

- 1 SC-1 Porometer - TCD Paleobotany lab ***[Porometry]***
- 1 Thermohygrometer - TCD Paleobotany lab ***[Porometry]***
- 1 Ceptometer - TCD Paleobotany lab ***[Porometry]***
- 1 GPS, GARMIN eTrex10 - TCD Paleobotany lab ***[Porometry, Herbarium]***
- 63 (21 trees x 3 leaves) Leaf tags - Any lightweight ribbon ***[Porometry]***
- 2 Plant presses, newspapers, cardboards - TCD Botany Dept teaching supplies ***[Elemental analysis/ Water Use Efficiency, Particulate matter analysis, Herbarium]***
- 189 Leaf tags for pressed plants - TCD Botany Dept ***[Elemental analysis/ Water Use Efficiency, Particulate matter analysis, Herbarium]***
- 126 Envelopes for preserving leaves - TCD palaeoecology lab ***[Elemental analysis/ Water Use Efficiency, Particulate matter analysis]***
- 100 (depending on dimension) Lint-free tissues ***[Particulate matter analysis]***
- 1 Single edged blade **[*Particulate matter analysis]***
- 63 SEM Stubs ***[Particulate matter analysis]***
- 63 Conductive tabs ***[Particulate matter analysis]***
- 1 Carbon paint ***[Particulate matter analysis]***
- Herbarium materials (sheets, stitching material, glue) ***[Herbarium]***
- Herbarium printed labels ***[Herbarium]***

Stomatal conductance

1 When

End of the growing season (July, not later than mid-August)

1.1 Leaf selection

1.1.1 Select **3 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 has to be horizontal

1.1.2 Per each leaf

- **Tag** leaf with a ribbon **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 GPS position of the tree (if new tree from the ones already used)

1.2 Porometry

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. Repeat approximately at the same time every day (to account the natural day-to-day variability in gs for each species under ambient conditions) following same order of trees and leaves measured

Note

Equipment-related:

- Calibrate every day or if temp changes of ca 15 degrees
- 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 the same position in the crown (e.g. base level, arm reach), to normalise for WUE and gs changes with tree height
- Measure always at the same position (e.g. right blade of leaf that face me)

During the measuremets:

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

1.2.2 At each tree measure and take note also of

- **Temp (°C), RH%** at each tree with thermo-hygrometer
- **Solar radiation** at each leaf with ceptometer's small sensor
- **Any other condition** met while taking the measurements (e.g. sudden wind, moving the leaves too much)

1.3 Download results

- Convert in .csv format
- Clean data (variable names lowercase, no spaces, keep only relevant info, add meatadata taken in the field i.e. temp, rh, notes). Final dataset should look like "*Expected results*" below

Expected result

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
	specimen_specie	leaf_id	lat	lon	date	time	gs	temp	sensor_serial_number	sensor_cal_number	leaf_sensor_rh	filter_sensor_rh	environment_rh	environment_temp	leaf_orientation	leaf_par	leaf_sun_exposure	leaf_appearance	notes
	Magnoliagrandiflora	MG01	53.3125	-6.2596666667	2022-08-12	9:18:03	172.5	22.1	LPS22 25	230360 4	45.1	21	70	21	W	40	Full sun	Healthy	Moved leaf horizontally, PAR retaken at 10:15 due to technical difficulties. The conditions had changed to shady during this time lapse.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
	Magnolia grandiflora	MG01	53.3125	-6.2596666667	2022-08-13	9:06:07	195.5	22.2	LPS2225	2203604	47.1	21.8	70.5	22.7	W	45	Sun flecks	Healthy	Moved leaf horizontally

1.4 Download metadata

- Take weather data from the garden MET station, readings from the 30 days before the monitoring (monitoring days included)
- Clean data. Final dataset should look like "*Expected result*" below

Expected result

	A	B	C	D	E	F	G	H	I	J	K
	doy	date	time	rh	temp	pressure	net_rad	par	wind_speed	wind_direction	precipitation
	152	2022-06-01	1:00:00	94.2	9.29	1012	NA	0.032	0.079	233.4	NA
	152	2022-06-01	1:30:00	94.7	9.26	1012	NA	0.023	0.129	176.1	NA

Leaf Area Index

2 When

During porometry, after taking stomatal conductance readings. It may not be possible to take LAI for each tree, depending on their local conditions (e.g. for trees that are shaded by other trees)

2.1 LAI readings

TBD

Elemental analysis/ Water Use Efficiency

3 **When**

Immediately after the last day of porometry

Collection needs to be after a period of 5 to 10 days of no rain and low winds, to avoid particulates to being washed away

3.1 Leaf selection

Same 3 leaves x tree **used for porometry**

3.2 Leaf collection and dehydration

2.2.1 **Pick** leaves and **tag** them with the same code used previously
2.2.2 **Press in plant press** for approx. 2 weeks, changing newspapers regularly ***or*** **Oven dry** at 50-60 °C for 2 days
2.2.3 **Sort** dried leaves in separate envelopes reporting leaf ID

3.3 Elemental analysis in the lab

TBD

3.4 Water Use Efficiency

TBD

Particulate Matter analysis

4 **When**

Collection must be immediately after the last day of porometry, or soon after. It has to be after a period of 5 to 10 days of no rain and low winds, to avoid particulates to be washed away

4.1 Leaf selection

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

Note

Each leaf has to be
- Healthy, pest- and pathogen-free
- Collected at basal exterior canopy

4.2 Leaf collection and dehydration

3.2.1 **Pick** leaves and **tag** them with the same code used previously + progressive numbering (i.e. **sample ID** that will be used in Scanning Electron Microscopy (SEM) later)
3.2.2 **Wrap** leaves in lint-free tissue to avoid contamination from paper fibers or other sources while pressing
3.2.3 **Press in plant press** for approx. 2 weeks, changing newspapers regularly *or* leave the press in oven at 50-60 °C for 2 days. Leaves need to be pressed so that can be imaged correctly under the SEM later
3.2.4 **Sort** dried leaves in separate envelopes reporting leaf ID and sample ID, keeping them wrapped in lint free tissue

4.3 Samples preparation in the Centre for Microscopy and Analysis lab

3.3.1 Cut 1 sample of 0.5 x 0.5 cm ca from each leaf with single edged blade

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

3.3.2 Prepare SEM stubs with conductive tabs
3.3.3 Using tweezers, mount 1 sample x stub and press firmly with lint-free tissue
3.3.4 Paint the edges of the sample with carbon paint (pure graphene) to prevent curling while in the SEM
3.3.5 Mark the bottom of the stub with the sample ID

Note

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

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

3.3.7 Carbon coat samples at 20 nm thickness

4.4 Analyses using TESCAN MIRA3 TIGER SEM

3.4.1 Set microscope settings

Note

On the TIGER set
- HV 15
- speed 3
- WD 15

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

3.4.2 **Map** samples
For each sample, pick 3 sub-sample areas at random at low magnification and increase resolution at 800 X

For each sub-sample
- Take micrographs using Secondary Electron light (SE) (optional)
- Map samples with Energy Dispersive X-Ray Spectroscopy (EDS) using Back Scattered Electron light. Map will be performed using the integrated AZtec software
- Label sub-samples as A, B, C (so e.g. sample 1, will have 1A, 1B, 1C)

3.4.3 **Save project** in double copy

4.5 Analyses using offline computer

3.5.1 Download saved project and open with AZtec

3.5.2 Set **feature analysis settings**

Note

On "Detect feature settings" window

- Image scan size
- Dwell time 25 microsec
- Input signal BSE
- Number of frames 1
- Smallest feature size 1 pixel (2.58 micron from settings, 0.38 micron for current image)
- Normalise results

On "detection refinement" window

- gray levels filter: smooth filer, gaussian smooth filter
- Binary image filter: erosion filter, close filter (regulate intensity base on the image), hole fill

On "Acquire site settings" window

- classification scheme: Midori (TCBG monitoring)
- classification filter pm10, pm2.5
- quant setup: normalise results, element list: fixed list and current spectrum

For each sub-sample

3.5.2 Select PM on the sample surface by tresholding image to select features by their 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)

3.5.3 Adjust filter settings to better select PM (e.g. use erosion filer if feature is bigger than actual PM when observed in the background). If filter settings still highlight features not corresponding to any actual PM, manually delete the features that do not match

3.5.4 Extract data on highlighted features, showing morphological and chemical information as in "Expected result" below

3.5.5 Copy-paste data on spreadsheet

4.6 Download results

- Convert in .csv format
- Clean data. See "Expected results" below

Expected result

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
	specimen_specie	leaf_id	sample_id	notes	lat	lon	pm_id	pm_class	pm_area	pm_ecd	pm_perimeter	pm_shape	pm_count	o	na	mg	al	si	k	ca
	Magnoliagrandiflora	MG01	1A	NA	53.312525	-6.259666666667	1	PM2.5	0.3414722469	0.6593754438	2.339323557	1.2753086884	NA	NA	NA	NA	NA	NA	NA	NA
	Magnoliagrandiflora	MG01	1A	NA	53.312525	-6.259666666667	6	PM2.5	0.4552963292	0.7613811732	2.1841040073	0.8337630498	NA	NA	NA	NA	NA	NA	NA	NA

Herbarium sheets

5 **When**

Collection must happen immediately after the last day of porometry, or soon after. It must be in dry weather to avoid molds in specimens

5.1 Selection of the specimen

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

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

4.1.2 **Label** each specimen annotating species name

4.1.3 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
- **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

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

5.2 Preparation of specimens

4.2.1 **Dehydrate plants** in plant press 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 of fruit in a way that they do not overlap
- Arrange so that specimen shows both sides of leaves e.g. turning one leaf to show the back
- If the specimen is too big for the press, bend it

4.2.2 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 that could contaminate the specimen that are already in the Herbarium.

5.3 Making of herbarium labels

Print labels with information noted in the field

Note

Tip

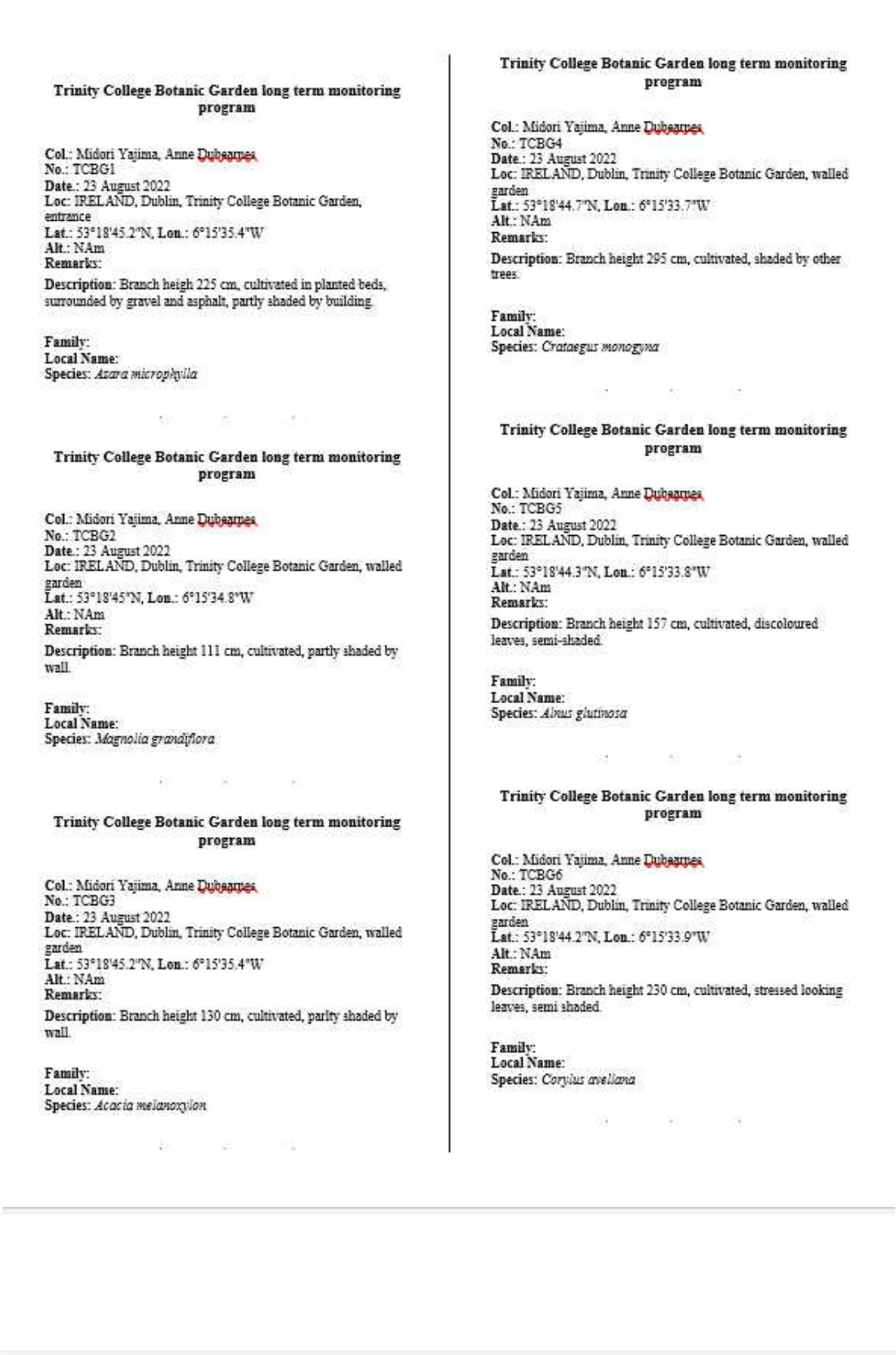
Copy information in a spreadsheet as it follows:

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	A
GLOBAL_UNIQUE_IDENTIFIER	HERBARIUM	TITLE	COLLECTOR	ADDITIONAL_COLLECTOR	COLLECTOR_NUMBER	DATE_COLLECTED	LOCAL_NAME	FAMILY	GENUS	SPECIES	AUTHOR_OF_SPECIES	INFRASPECIFIC_RANK	INFRASPECIFIC_EPITHET	AUTHOR_OF_INFRASPECIFIC_RANK	COUNTRY	STATE_PROVINCE	COUNTY	LOCALITY	IMAGE_URL	RELATED_INFORMATION	LAT_DEGREE	LAT_MINUTE	LAT_SECOND	LAT_FLAG	LON_DEGREE	LON_MINUTE	L
	Tri																										

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	A
		nity College Botanic Garden long term monitoring program		Midori Yajima, Anne Dubeares		TCBG1	23-Aug-22			Azara	microphylla					Ireland	Dublin		Trinity College Botanic Garden, entrance			53	18	45.2	N	6	15	3 5 4

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	A
		Trinity College Botanic Garden long term monitoring program		Midori Yajima, Anne Dubeaarnes		TCBG2	23-Aug-22			Magnolia	grandiflora					Ireland	Dublin		Trinity College Botanic Garden, walled garden			53	18	45	N	6	15	3 4 8

And use the herblabel R package (using "KUN") to print labels instead of creating them one by one. Final result should look like



Trinity College Botanic Garden long term monitoring program

Col.: Midori Yajima, Anne Dubearnes
No.: TCBG1
Date.: 23 August 2022
Loc: IRELAND, Dublin, Trinity College Botanic Garden, entrance
Lat.: 53°18'45.2"N, **Lon.**: 6°15'35.4"W
Alt.: NAm
Remarks:
Description: Branch heigh 225 cm, cultivated in planted beds, surrounded by gravel and asphalt, partly shaded by building.

Family:
Local Name:
Species: *Azara microphylla*

Trinity College Botanic Garden long term monitoring program

Col.: Midori Yajima, Anne Dubearnes
No.: TCBG2
Date.: 23 August 2022
Loc: IRELAND, Dublin, Trinity College Botanic Garden, walled garden
Lat.: 53°18'45"N, **Lon.**: 6°15'34.8"W
Alt.: NAm
Remarks:
Description: Branch height 111 cm, cultivated, partly shaded by wall.

Family:
Local Name:
Species: *Magnolia grandiflora*

Trinity College Botanic Garden long term monitoring program

Col.: Midori Yajima, Anne Dubearnes
No.: TCBG3
Date.: 23 August 2022
Loc: IRELAND, Dublin, Trinity College Botanic Garden, walled garden
Lat.: 53°18'45.2"N, **Lon.**: 6°15'35.4"W
Alt.: NAm
Remarks:
Description: Branch height 130 cm, cultivated, parlty shaded by wall.

Family:
Local Name:
Species: *Acacia melanoxylon*

Trinity College Botanic Garden long term monitoring program

Col.: Midori Yajima, Anne Dubearnes
No.: TCBG4
Date.: 23 August 2022
Loc: IRELAND, Dublin, Trinity College Botanic Garden, walled garden
Lat.: 53°18'44.7"N, **Lon.**: 6°15'33.7"W
Alt.: NAm
Remarks:
Description: Branch height 295 cm, cultivated, shaded by other trees.

Family:
Local Name:
Species: *Crataegus monogyna*

Trinity College Botanic Garden long term monitoring program

Col.: Midori Yajima, Anne Dubearnes
No.: TCBG5
Date.: 23 August 2022
Loc: IRELAND, Dublin, Trinity College Botanic Garden, walled garden
Lat.: 53°18'44.3"N, **Lon.**: 6°15'33.8"W
Alt.: NAm
Remarks:
Description: Branch height 157 cm, cultivated, discoloured leaves, semi-shaded.

Family:
Local Name:
Species: *Alnus glutinosa*

Trinity College Botanic Garden long term monitoring program

Col.: Midori Yajima, Anne Dubearnes
No.: TCBG6
Date.: 23 August 2022
Loc: IRELAND, Dublin, Trinity College Botanic Garden, walled garden
Lat.: 53°18'44.2"N, **Lon.**: 6°15'33.9"W
Alt.: NAm
Remarks:
Description: Branch height 230 cm, cultivated, stressed looking leaves, semi shaded.

Family:
Local Name:
Species: *Corylus avellana*

5.4 Making of herbarium sheets

4.3.1 **Mount specimens** on herbarium sheets, making sure to

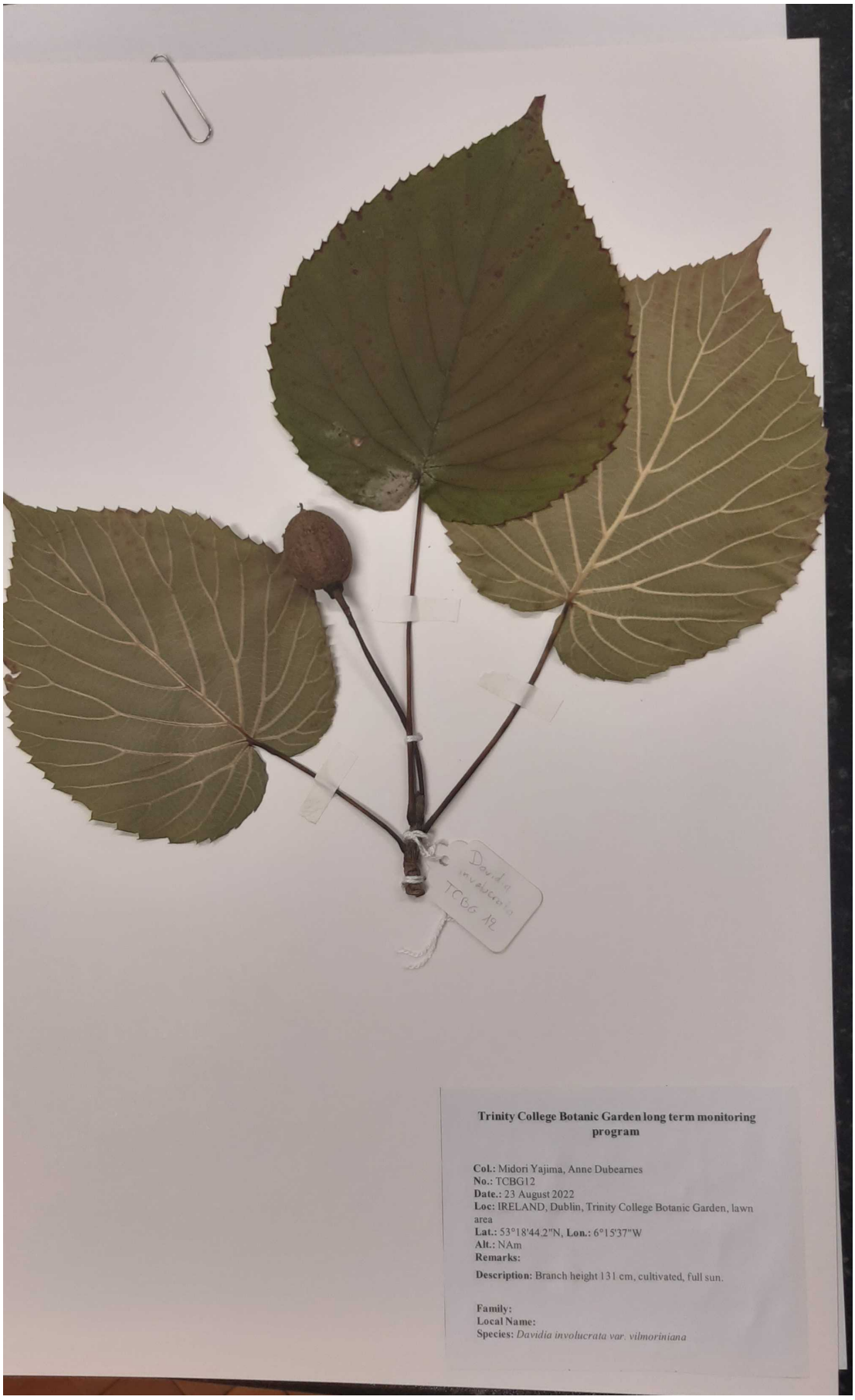
- Stitching them instead of using glue. Use glue only if necessary (e.g. specimen is too

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

4.3.2 Store herbarium sheet in dedicated box in Trinity Herbarium

Expected result


Davidia involucrata
TCBG 12
Trinity College Botanic Garden long term monitoring program
Col.: Midori Yajima, Anne Dubearnes
No.: TCBG12
Date.: 23 August 2022
Loc: IRELAND, Dublin, Trinity College Botanic Garden, lawn area
Lat.: 53°18'44.2"N, Lon.: 6°15'37"W
Alt.: NAm
Remarks:
Description: Branch height 131 cm, cultivated, full sun.
Family:
Local Name:
Species: Davidia involucrata var. vilmoriniana

Upload data on Dryad

6 TBD