



pollen 
PALS

Biotic and abiotic effects
on pollen production and
allergenicity of birch and
related health impacts

Instruction manual for
collecting pollen and leaf
samples within the DFG
project *pollenPALS*



Fifth Edition | 2023



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pollenPALS@ku.de

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Foreword



Dear supporters of our project,

First of all, I would like to thank you for your previous help in the pollenPALS project. With the analysis of your birch catkins, we were able to compile a distinctive data treasure. By evaluating the data from previous years, we have shown that pollen production is on average higher at warmer sites. This assertion is particularly important for estimating the future effects of climate change.

After the official end of the project on December 31, 2022, we are now submitting our final report in the form of a follow-up proposal and hope for a positive assessment in the course of the year. In this proposal, we would like to concentrate on the International Phenological Gardens again, since the work has led to many new questions that now need to be answered. These includes, for example, the investigation of regional differences in masting behavior of birch. However, a substantial benefit arises from the fact that, during the follow-up project, we can produce a small time series.

This year, we ought to continue the monitoring by self-funding so that we could ensure no gaps in this time series. Hence, we ask for your further support again. We assure for the expense allowance and will send you all the necessary materials for sampling and shipping as we did in previous years.

The sampling procedure is not changed compared to the previous year and you will find all the important information again in this brochure (page 10 ff).

I would like to thank you very much on behalf of the entire project team for your help!

Susanne Jochner-Oette

The procedure for collecting and drying catkins was not changed compared to the previous year.

Additional information can be found on pages 10 ff and in the attached leaflets.

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Changes compared to the previous year at a glance

... at a
glance

The procedure for collecting and drying catkins was not changed compared to the previous year.

Additional information can be found on pages 10 ff and in the attached leaflets.

BIRCH POLLEN

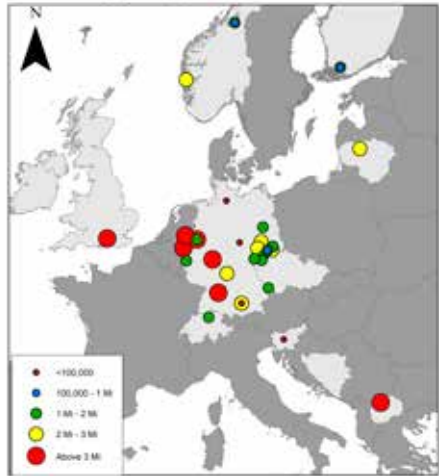
pollen
PALS
2022

Review 2022 and first findings

In total, 31 IPGs participated in our 2022 sampling campaign. We received samples from 29 IPGs for stage 51/52, from 20 IPGs for stage 60 and 29 IPGs contributed with leaf samples. The following 11 countries supported our project: Bosnia and Herzegovina, Czech Republic, Finland, Germany, Ireland, Lithuania, Macedonia, Norway, Slovenia, Switzerland, and the UK.

These samples are now in the freezers of our laboratories and are waiting to be evaluated in the follow-up project. Therefore, the following results refer to the analysis of the data from the years 2019 to 2021.

Most of the trees were characterized by a diverse pollen production at catkin level with maximum values of 7×10^6 pollen grains (average 2×10^6 pollen grains). We found a significant and positive correlation of pollen production per catkin with preceding annual temperatures (correlation coefficient $r = 0.52$, $p < 0.001$). Partial regression analyses revealed that other variables such as altitude of the site and air pollution were also significantly associated to pollen production, but the importance of these factors differed across years. Infections with Cherry leaf roll virus were not related to pollen production. However, we observed that a higher pollen production was linked to higher allergenicity values.



Average pollen production per catkin (2019–2021) at the IPG sites

Our findings indicate that global warming may exert further influences on pollen production of birch and therefore on human health. Establishing a long-term monitoring of pollen production would be highly desirable for investigating confounding masting effects.

Specifications pollenPALS

Keywords and highlights

	<i>Allergic potential across Europe</i>	<i>... from Scandinavia to Macedonia</i>
<i>Species: Downy birch (<i>Betula pubescens</i>)</i>		<i>Analysis of biotic effects</i>
<i>... and abiotic effects</i>	<i>... on pollen production and pollen allergenicity</i>	



Project description

Although higher temperatures were often found to increase pollen production and allergenicity, several studies reported decreased quantities of pollen and allergen content under warmer conditions. Up to now, little is known about additional biotic and abiotic factors that might alter these pollen characteristics. There might exist a remarkable difference in the impact of pollen on human beings depending on the overall production and allergenic potential of pollen.

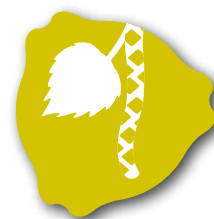
In this study, we focus on the most important allergenic tree species in northern, central, and eastern Europe: birch (*Betula* spp.). **The major novelty is the analysis of biotic and abiotic impacts on pollen of cloned birch individuals free from genetic differences in their natural environment across International Phenological Gardens in Europe.** Besides the investigation of abiotic factors (e.g., air temperature, relative humidity, air pollutants) influencing pollen production and allergen content, we also focus on biotic factors such as virus infections (Cherry leaf roll virus) or the pollen-associated microbiome and the *in vivo* relevance of the pollen's allergenicity using skin prick tests.

A greater knowledge gained by our study allows the prediction of future alterations under climate change conditions in more detail. An interdisciplinary collaboration between scientists of landscape ecology, phytomedicine and environmental medicine shall answer how biotic and abiotic factors impact pollen production and allergenicity and finally the allergic reactions in patients.












Information

- Studied species:
Downy birch (Betula pubescens)
- 47 IPG sites across Europe
- Peculiarity of IPGs:
 - » The network includes stations from different climatic zones.



- » The major novelty is the analysis of environmental impacts on pollen in the natural environment across Europe free from genetic differences.

Phenological stages

Code	Visualization	Description	
50		Dormancy: catkins still brown and not stretched	
51 or 52		First green spots visible; although negligible stretching	<i>used for the analyses of pollen production</i>
		Catkins increase in length and show green expansion cracks	
55		Enhanced expansion cracks through further increase in length	
59		Maximal lengthening of catkins; anthers clearly visible	
60 or 61		First catkins emit pollen (sporadically)	<i>used for the analyses of pollen allergenicity</i>
		Beginning of flowering : catkins emit pollen spontaneously when touching them	
63		more than 10 % but less than 50 % of the catkins emit pollen	
65 to 69		Full flowering (50%) to the end of flowering	



Stage
51/52
or 60/61

Stage
51/52
or 60/61

- Pollen production will be assessed with catkins exhibiting the phenological stage 51/52. If catkins already emitted pollen (stage 60), the estimation of pollen production is not practicable anymore. Stage 60 and 61 however, should be used for the analysis of pollen allergenicity.



- Thus, catkins should be harvested at two time points:
AT STAGE 51/52 AND 60/61 AND TWO PARCELS HAVE TO BE SENT BACK.

Informations

- **Stage 51/52:** Catkins exhibit green spots. They are slightly stretched and can show first expansion cracks.
- Pollen is only released at a later stage (60).
Test on pollen release:
Tap catkins on black paper in order to see if yellowish pollen is released. By touching them pollen should be released spontaneously.

This is the right collection stage.

Sampling ONE at stage 51/52

• Samples for the estimation of pollen production:

10 catkins from the **northern, eastern, southern and western side** of the tree are harvested from different branches and stored in the enclosed paper bags.

The sampling should be achieved at 1.5 to 2 m above ground (e.g. also with an extendable handle and with a ladder).

▶ *If only one site, or not all sites are accessible, this has to be noted in the form.*

• **Please use the form on the next page which should be sent back to us.**

• How many catkins are needed:

▶ **10 catkins from the northern, eastern, southern and western side of the tree (40 in total at stage 51/52)**

Remark: Should you have missed stages 51/52 but the catkins do not flower yet (stage 60), we would be grateful for sending us catkins at stage 55 or 59 (although the estimation of pollen production might be not reliable).

The catkins should be directly harvested in the paper bags: e.g. after sampling, the bags should immediately be inserted in the parcel and sent back to us via express delivery.

In addition to the expense allowance, we will cover all costs related to express delivery.



Counting of catkins in a cube

Use a pocket rule for counting the number of birch catkins within a defined volume (50x50x50 cm³). This measure is important for the estimation of the pollen production of the whole tree.

Hold the rule (see picture) in ca. 1.5 m height. To count the number of catkins, please select random tree parts and sides, not deliberately those that are the most abundant in catkins. Note the number of catkins in the form.

Please fill this form **ONE** (front and back):

- Ballyhaise College
- Bergen-Fana
- Botanical Garden
Siauliai Uni.
- Dublin-Nation.
Botan. Gardens
- Edinburgh/Inverleith
- Evora
- Glenveagh National Park
- Kerry-Valentia-Observatory
- Ljubljana
- Logan
- London-Farnham-Alice Holt
- Markree Castle
- Millstreet Country Park
- Mostar
- Pierroton
- Praha-Doksany
- Praha-Kostelec
- RBG-Edinburgh/Dawyck
- Salzburg
- Recklinghausen
- Roßla
- Sarajevo
- Sarajevo-Ivan Sedlo
- Skopje
- Trondhjem-Sjordal-Kvithamar
- Turku-Pilkiö-Yltöinen
- Wexford-Johnstown Castle
- Zürich-Birmensdorf

*Information
on your location*

Contact person on site

*Betula pubescens
Tree 1/2*

Contact person on site

Name: _____

Email: _____

The **yearly expense allowance (50 €)** should be transferred to the following bank account:
(to be filled only in the case of changes compared to 2021)

Account holder: _____

BIC: _____

IBAN: _____

FORM ONE

Individual 2

Age _____ Year of planting _____ * see page 15

Trunk circumference _____ Tree height _____

Crown height _____

Crown diameter N-S direction: _____

Crown diameter W-E direction: _____

Shade Half-shade Sun

Visual rating: tree has a few , much or very much catkins

Number of catkins within a cube (50 x 50 x 50 cm³) _____

Sampling was achieved at stages 51/52

Divergent stage _____

Sampling was possible at all sites or only at the side(s) _____

Height above ground of sampled catkins _____

Date of sampling _____

Weather while sampling _____

Is an illness detectable? _____

Other peculiarities (e.g. frost cracks, forks, cuts, mistletoes, tree fungus etc.): _____

Individual 1

Age _____ Year of planting _____ * see page 15

Trunk circumference _____ Tree height _____

Crown height _____

Crown diameter N-S direction: _____

Crown diameter W-E direction: _____

Shade Half-shade Sun

Visual rating: tree has a few , much or very much catkins

Number of catkins within a cube (50 x 50 x 50 cm³) _____

Sampling was achieved at stages 51/52

Divergent stage _____

Sampling was possible at all sites or only at the side(s) _____

Height above ground of sampled catkins _____

Date of sampling _____

Weather while sampling _____

Is an illness detectable? _____

Other peculiarities (e.g. frost cracks, forks, cuts, mistletoes, tree fungus etc.): _____



More Information

For simplifying the procedure of sampling catkins, we provide a **video** at: https://youtu.be/vlzO_17ZTog.

As phenological observer you will know exactly about the flowering dates. However, you might not explicitly note the **duration of flowering** (from stage 60 to 69). Thus, we would like to ask you also to note this duration. We will request this information at a later stage via email since the catkins and forms should be returned before the end of flowering.

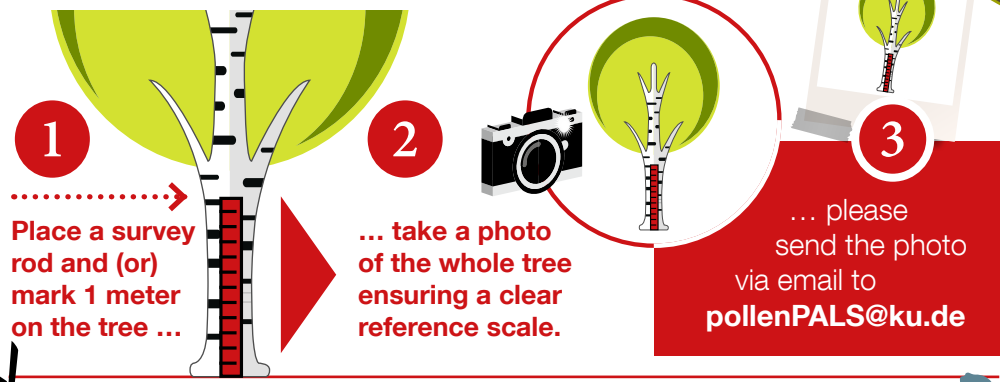
Flowering duration (please insert dates)

Date stage 60 _____ (first pollen is released)

Date stage 69 _____ (catkins do not emit pollen anymore)



* Estimation of tree height (if still pending)



Sampling TWO at stage 60/61

- **Composite sample for the estimation of pollen allergenicity:**

The sample should be a composite sample, randomly selected (e.g. also with an extendable handle and with a ladder).

► *If only one site, or not all sites are accessible, this has to be noted in the form.*

- **Please** use the form on the next page which should be sent back to us.

- **Please** collect the catkins with the enclosed gloves in order to avoid a contamination and use the mouth protection.

- **How many catkins are needed:**

► **preferably 150–200 catkins**
at stage 60/61



Attention!

The sampling should not be done by people allergic against birch pollen. In any case sampling should be done using gloves and mouth protection in order to avoid an irritation and sensibilisation. After sampling a shower is recommended.

The catkins should be directly harvested in the dedicated paper bags. After the sampling, the catkins should be dried on a DIN A3 paper for two days (three days for wet catkins) at room temperature. Afterwards, the catkins should be placed in the paper bag. The bag should be sealed with an adhesive tape firmly and placed in a plastic bag (Zip Lock) between silica gel. The catkins should be sent via express delivery immediately. We will cover all costs related to the shipment.



Please dry the catkins before shipping.

in the laboratory of Humboldt-Universität zu Berlin



If you are unsure about the correct sampling stage, you can contact us via

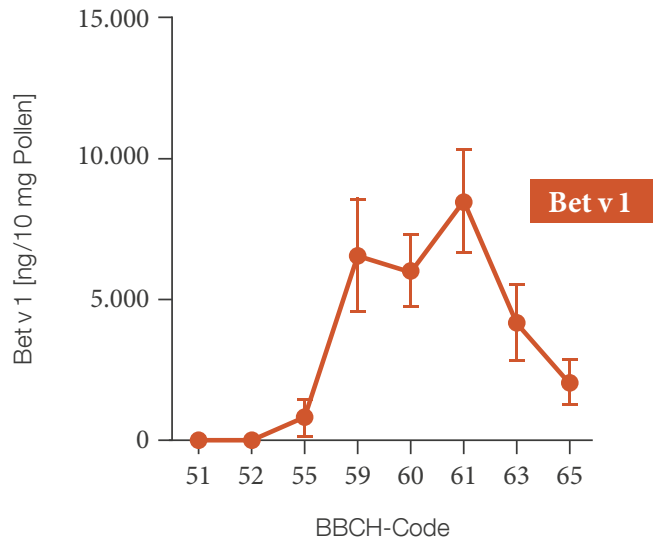
pollenPALS@ku.de

Background information

Why is the assessment of allergenicity most meaningful at stage 60/61?

Bet v 1 is the major allergen of birch and belongs to the stress-induced plant proteins. The allergen amount is not constant but varies in the course of the flowering period.

Bet v 1 levels only increase shortly before pollen release and decrease remarkably from stage 63 onwards. Comparable results can only be achieved if the catkins were harvested at a similar phenological stage and pollen of highest allergenicity can be extracted. **Thus, please only harvest catkins characterized by stage 60/61.** If you send catkins exhibiting an earlier or later stage, please inform us about that.



Dependency of Bet v 1 and maturity of catkins. The figure shows mean values ± standard errors derived from five birches whose catkins were harvested at different phenological stages.

Modified after: Beck I, Jochner S, Gilles S, McIntyre M, Buters JTM, Schmidt-Weber C, Behrendt H, Ring J, Menzel A, Traidl-Hoffmann C (2013) High environmental ozone levels lead to enhanced allergenicity of birch pollen. PLOS ONE, 8(11), e80147. doi: 10.1371/journal.pone.0080147.

Please fill this form *TWO* (front and back):

- Ballyhaise College
- Bergen-Fana
- Botanical Garden Siauliai Uni.
- Dublin-Nation. Botan. Gardens
- Edinburgh/Inverleith
- Evora
- Glenveagh National Park
- Kerry-Valentia-Observatory
- Ljubljana
- Logan
- London-Farnham-Alice Holt
- Markree Castle
- Millstreet Country Park
- Mostar
- Pierroton
- Praha-Doksany
- Praha-Kostelec
- RBG-Edinburgh/Dawyck
- Salzburg
- Recklinghausen
- Roßla
- Sarajevo
- Sarajevo-Ivan Sedlo
- Skopje
- Trondhjem-Sjordal-Kvithamar
- Turku-Pilkiö-Yltöinen
- Wexford-Johnstown Castle
- Zürich-Birmensdorf

Information on your location

Contact person on site

Betula pubescens
Tree 1/2

Contact person on site

Name: _____

Email: _____

The **yearly expense allowance (50 €)** should be transferred to the following bank account:
(to be filled only in the case of changes compared to 2021)

Account holder: _____

BIC: _____

IBAN: _____

FORM TWO

Individual 2

Age _____ Year of planting _____ * see page 15

Trunk circumference _____ Tree height _____

Crown height _____

Crown diameter N-S direction: _____

Crown diameter W-E direction: _____

Shade Half-shade Sun

Visual rating: tree has a few , much or very much catkins

Number of catkins within a cube (50 x 50 x 50 cm³) _____

Sampling was achieved at stages 60/61

Divergent stage _____

Sampling was possible at all sites or only at the side(s) _____

Height above ground of sampled catkins _____

Date of sampling _____

Weather while sampling _____

Is an illness detectable? _____

Other peculiarities (e.g. frost cracks, forks, cuts, mistletoes, tree fungus etc.): _____

Individual 1

Age _____ Year of planting _____ * see page 15

Trunk circumference _____ Tree height _____

Crown height _____

Crown diameter N-S direction: _____

Crown diameter W-E direction: _____

Shade Half-shade Sun

Visual rating: tree has a few , much or very much catkins

Number of catkins within a cube (50 x 50 x 50 cm³) _____

Sampling was achieved at stages 60/61

Divergent stage _____

Sampling was possible at all sites or only at the side(s) _____

Height above ground of sampled catkins _____

Date of sampling _____

Weather while sampling _____

Is an illness detectable? _____

Other peculiarities (e.g. frost cracks, forks, cuts, mistletoes, tree fungus etc.): _____



Please fill this form **THREE** (front and back) :

Please help us identify virus infected trees by sending leaves with (and without) symptoms as described on the next page.

Virus symptoms such as changes in colour and form of the leaves can typically appear on leaves of single branches. Please note on the form (next page) if you already observed such symptoms and inform us on any previously observed symptoms in the sampled trees.

SAMPLING

If no symptoms are visible, please cut small twigs from each cardinal direction. If symptoms are visible, we need a sample with leaves displaying these symptoms and a control samples without symptoms from another part of the same tree. Cut small twigs with ca. 5–10 leaves. These should then be wrapped in damp paper and sealed in the enclosed plastic bags (Zip Lock), labelled and packed in an already addressed envelope to Berlin.

TRANSPORT

Please choose express delivery for which we will cover the costs.

STORAGE

If you are unable to send samples immediately please store them at 4 ° C, for example in the fridge. Please do not freeze samples as after thawing, plant material is no longer suitable for testing.

SAMPLING TIME

In the early summer, after the entire leaf is completely developed.

Plant viruses are found in birch trees throughout the world. Further steps to clarify the importance of viruses and the role they play can be achieved with your help. For our research project, we require birch leaves of virus-infected trees, but also samples from non-infected birches for control purposes. These samples will be studied further in a laboratory.



FORM THREE



Symptoms of leaves

Did you observe those or similar symptoms in the leaves of your trees (birch) in the Phenological Gardens?

Please make a note of the tree number and a cross below!

If you found different symptoms, please describe them.

FORM THREE

Interference of chlorotic venation and mottling



Vein chlorosis



Leaf edge chlorosis



Intercostal chlorosis



Leaf chlorosis



Leaf chlorosis



Mottling



Weak mottling



Blistering and mottling



Mottling and veinnetting



Mottling and in parts mosaic



Irregular chlorotic spotting



Oak leaf pattern



Variegation related



Line pattern related



Chlorotic line pattern (white)



Chlorotic line pattern (yellow)



Chlorotic spots



Necrosis of leaves



Leaf deformation and leaf chlorosis



Leaf deformation, leaf curl and chlorosis



Leaf curl and necrosis



Defoliation



Small leaves

Thank you very much for supporting our project!



Picture: Miriam Sieverts and Verena Wörl
Map: colourbox.de (modified and supplemented 2021)

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