



Supplement of

High interspecific variability in ice nucleation activity suggests pollen ice nucleators are incidental

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Table S1: Details of pollen collections and classifications.

Species	Family	Order	Location*	Date	T_{50} / conc.	T_{rep}	Sub-division	Biome	Poll. season	Poll. method	Elevation category	Low elevation	High elevation	Desicc. tolerance
<i>Abies balsamea</i> (L.) Mill.	Pinaceae	Pinales	Wakehurst Place	09/04/22	-12.6 °C / 2 wt%	-13.6 °C	Gymnosperm	Temperate	Spring	Wind	Moderate	0	1900	--
<i>Acer pseudoplatanus</i> L.	Sapindaceae	Sapindales	Podivín, Czech Republic	22/04/18- 23/04/18	-16.6 °C / 2 wt%	-17.7 °C	Angiosperm	Temperate	Spring	Animal	Moderate	200	1500	DT
<i>Alnus glutinosa</i> (L.) Gaertn.	Betulaceae	Fagales	**	**	-16.3 °C / 2 wt%	-17.7 °C	Angiosperm	Temperate	Spring	Wind	Moderate	0	1800	DS
<i>Amaranthus hybridus</i> L.	Amaranthaceae	Caryophyllales	Třebíč, Czech Republic	09/08/22- 07/09/22	-17.5 °C / 2 wt%	-19.6 °C	Angiosperm	Seasonally dry tropical	Summer	Wind	Low	0	300	--
<i>Araucaria araucana</i> (Molina) K.Koch	Araucariaceae	Araucariales	Kew Gardens	02/06/21	-16.6 °C / 2 wt%	-17.4 °C	Gymnosperm	Temperate	Summer	Wind	Moderate	600	2000	--
<i>Arundo formosana</i> Hack.	Poaceae	Poales	Kew Gardens	01/09/21	-21.0 °C / 0.2 wt%	-19.6 °C	Angiosperm	Subtropical	Summer	Wind	Moderate	0	1800	--
<i>Betula alleghaniensis</i> Britton	Betulaceae	Fagales	Wakehurst Place	03/04/22	-9.0 °C / 2 wt%	-10.7 °C	Angiosperm	Temperate	Spring	Wind	Moderate	300	1900	DT
<i>Betula x caerulea</i> Blanch.	Betulaceae	Fagales	Wakehurst Place	03/04/22	-9.0 °C / 2 wt%	-11.4 °C	Angiosperm	Temperate	Spring	Wind	Low	500	500	--
<i>Betula ermanii</i> Cham.	Betulaceae	Fagales	Wakehurst Place	09/04/22	-15.2 °C / 2 wt%	-16.8 °C	Angiosperm	Temperate	Spring	Wind	High	1600	2900	--
<i>Betula pendula</i> Roth	Betulaceae	Fagales	České Budějovice, Czech Republic	08/04/20- 10/04/20	-16.0 °C / 2 wt%	-16.5 °C	Angiosperm	Temperate	Spring	Wind	Moderate	0	1500	--
<i>Betula utilis</i> subsp. <i>jacquemontii</i> (Spach) Kitam.	Betulaceae	Fagales	Wakehurst Place	03/04/22	-8.6 °C / 2 wt%	-9.7 °C	Angiosperm	Temperate	Spring	Wind	High	2700	3900	--
<i>Camellia reticulata</i> Lindl.	Theaceae	Ericales	Kew Gardens	02/12/21	-21.9 °C / 0.2 wt%	-20.8 °C	Angiosperm	Subtropical	Autumn/ Winter	Animal	High	1800	2500	--
<i>Camellia saluenensis</i> Stapf ex Bean	Theaceae	Ericales	Kew Gardens	02/12/21	-20.5 °C / 0.2 wt%	-19.3 °C	Angiosperm	Temperate	Autumn/ Winter	Animal	High	1900	3200	--
<i>Carpinus betulus</i> L.	Betulaceae	Fagales	Wakehurst Place	03/04/23	-15.8 °C / 2 wt%	-17.3 °C	Angiosperm	Temperate	Spring	Wind	Moderate	0	1800	--
<i>Carpinus cordata</i> Blume	Betulaceae	Fagales	Wakehurst Place	04/04/23	-16.0 °C / 2 wt%	-18.4 °C	Angiosperm	Temperate	Spring	Wind	High	200	2500	--
<i>Cedrus atlantica</i> (Endl.) Manetti ex CarriPre	Pinaceae	Pinales	Kew Gardens	02/12/21	-8.8 °C / 2 wt%	-10.6 °C	Gymnosperm	Temperate	Autumn/ Winter	Wind	High	1300	2600	--
<i>Cedrus atlantica</i> f. <i>glauca</i> (CarriPre) Beissn.	Pinaceae	Pinales	Kew Gardens	02/12/21	-12.9 °C / 2 wt%	-16.8 °C	Gymnosperm	Temperate	Autumn/ Winter	Wind	High	1300	2600	--
<i>Cedrus deodara</i> (Roxb. ex D.Don) G.Don	Pinaceae	Pinales	Leamington Spa	05/12/21	-15.2 °C / 2 wt%	-17.4 °C	Gymnosperm	Temperate	Autumn/ Winter	Wind	High	1200	3000	--
<i>Cestrum fasciculatum</i> (Schltdl.) Miers	Solanaceae	Solanales	Kew Gardens Glasshouses	02/12/21	-23.6 °C / 0.2 wt%	-21.0 °C	Angiosperm	Seasonally dry tropical	Summer	Animal	Low	0	1000	--
<i>Clerodendrum speciosissimum</i> Jacob-Makoy	Lamiaceae	Lamiales	Kew Gardens Glasshouses	01/09/21	-21.5 °C / 0.2 wt%	-20.0 °C	Angiosperm	Wet tropical	Summer	Animal	Moderate	0	1500	--

<i>Corylus avellana</i> L.	Betulaceae	Fagales	**	**	-17.9 °C / 2 wt%	-19.1 °C	Angiosperm	Temperate	Autumn/ Winter	Wind	Low	0	1020	DS
<i>Crocus vernus</i> (L.) Hill	Iridaceae	Asparagales	Leamington Spa	25/02/21	-12.1 °C / 2 wt%	-13.1 °C	Angiosperm	Temperate	Spring	Animal	Moderate	600	1600	--
<i>Cupressus sempervirens</i> L.	Cupressaceae	Cupressales	Alcoy, Spain	27/02/21	-19.3 °C / 2 wt%	-20.6 °C	Gymnosperm	Temperate	Spring	Wind	Low	350	1100	--
<i>Cynosurus cristatus</i> L.	Pooideae	Poales	Wakehurst Place	30/06/21	-17.7 °C / 0.2 wt%	-17.1 °C	Angiosperm	Temperate	Summer	Wind	Low	0	800	--
<i>Dactylis glomerata</i> L.	Pooideae	Poales	Cheb, Czech Republic	15/06/21- 17/06/21	-17.1 °C / 2 wt%	-18.9 °C	Angiosperm	Temperate	Summer	Wind	Moderate	0	2400	--
<i>Encephalartos equatorialis</i> P.J.H.Hurter	Zamiaceae	Cycadales	Nong Nooch Bot. Garden, Thailand	11/03/19	-19.7 °C / 0.2 wt%	-18.7 °C	Gymnosperm	Seasonally dry tropical	Summer	Animal	Moderate	1000	1000	--
<i>Erica multiflora</i> L.	Ericaceae	Ericales	Alcoy, Spain	27/02/21	-15.9 °C / 2 wt%	-17.3 °C	Angiosperm	Subtropical	Autumn/ Winter	Animal	Low	0	1200	--
<i>Hedychium coronarium</i> J.Koenig	Zingiberaceae	Zingiberales	Kew Gardens Glasshouses	01/09/21	-19.3 °C / 0.2 wt%	-17.5 °C	Angiosperm	Subtropical	Summer	Animal	High	0	2500	--
<i>Helianthus annuus</i> L.	Asteraceae	Asterales	Břeclav, Czech Republic	15/06/18- 27/06/18	-16.0 °C / 2 wt%	-21.3 °C	Angiosperm	Temperate	Summer	Animal	Moderate	0	1500	DT
<i>Hordeum vulgare</i> L.	Poaceae	Poales	České Budějovice, Czech Republic	18/06/18	-20.0 °C / 2 wt%	-21.2 °C	Angiosperm	Temperate	Summer	Wind	Moderate	0	1500	--
<i>Hymenocallis littoralis</i> (Jacq.) Salisb.	Amaryllidaceae	Asparagales	Kew Gardens Glasshouses	01/09/21	-17.33 °C / 0.2 wt%	-8.9 °C	Angiosperm	Wet tropical	Summer	Animal	Moderate	0	1400	--
<i>Juglans regia</i> L.	Juglandaceae	Fagales	Břeclav, Czech Republic	22/04/16- 23/04/16	-18.9 °C / 2 wt%	-19.5 °C	Angiosperm	Temperate	Spring	Wind	Moderate	0	2000	DS
<i>Musa rubra</i> Wall. ex Kurz	Musaceae	Zingiberales	Kew Gardens Glasshouses	01/09/21	-23.8 °C / 2 wt%	-26.7 °C	Angiosperm	Subtropical	Year round	Animal	Moderate	0	2100	DS
<i>Narcissus papyraceus</i> subsp. <i>polyanthos</i> (Loisel.) Asch. & Graebn.	Amaryllidaceae	Asparagales	Kew Gardens Glasshouses	02/12/21	-23.8 °C / 0.2 wt%	-21.2 °C	Angiosperm	Subtropical	Autumn/ Winter	Animal	Moderate	0	1200	--
<i>Nymphaea</i> 'Kew's Stowaway Blues'	Nymphaeaceae	Nymphaeales	Kew Gardens Glasshouses	01/09/21	-20.8 °C / 2 wt%	-22.5 °C	Angiosperm	Cosmopolitan	Summer	Animal	***	***	***	--
<i>Ostrya carpinifolia</i> Scop.	Betulaceae	Fagales	Wakehurst Place	04/04/23	-14.5 °C / 2 wt%	-15.4 °C	Angiosperm	Temperate	Spring	Wind	Moderate	0	1300	DS
<i>Picea abies</i> (L.) H.Karst.	Pinaceae	Pinales	Velechvín, Czech Republic	06/05/17	-17.8 °C / 2 wt%	-20.7 °C	Gymnosperm	Temperate	Summer	Wind	High	0	2400	DT
<i>Picea brachytyla</i> (Franch.) E.Pritz.	Pinaceae	Pinales	Kew Gardens	02/06/21	-15.7 °C / 2 wt%	-18.1 °C	Gymnosperm	Temperate	Summer	Wind	High	1500	3500	DT
<i>Pilgerodendron uviferum</i> (D.Don) Florin	Cupressaceae	Cupressales	Wakehurst Place	04/04/23	-17.6 °C / 2 wt%	-18.9 °C	Gymnosperm	Temperate	Spring	Wind	Moderate	0	1200	--
<i>Pinus contorta</i> var. <i>contorta</i>	Pinaceae	Pinales	Wakehurst Place	19/05/22	-18.2 °C / 2 wt%	-19.4 °C	Gymnosperm	Temperate	Summer	Wind	Low	0	610	DT
<i>Pinus coulteri</i> D.Don	Pinaceae	Pinales	Kew Gardens	02/06/21	-12.2 °C / 2 wt%	-12.7 °C	Gymnosperm	Subtropical	Spring	Wind	Moderate	150	2120	DT
<i>Pinus halepensis</i> Mill.	Pinaceae	Pinales	Alcoy, Spain	17/03/21	-16.6 °C / 2 wt%	-19.7 °C	Gymnosperm	Temperate	Spring	Wind	Moderate	250	2000	DT

<i>Pinus mugo</i> Turra	Pinaceae	Pinales	Warwick Univ. Campus	24/05/23	-7.6 °C / 2 wt%	-8.7 °C	Gymnosperm	Temperate	Spring	Wind	High	200	2700	DT
<i>Pinus ponderosa</i> Douglas ex C.Lawson	Pinaceae	Pinales	Lednice, Czech Republic	25/04/16- 01/05/16	-20.6 °C / 2 wt%	-21.6 °C	Gymnosperm	Temperate	Spring	Wind	High	0	3050	DT
<i>Plantago lanceolata</i> L.	Plantaginaceae	Lamiales	Wakehurst Place	30/06/21	-18.1 °C / 0.2 wt%	-17.8 °C	Angiosperm	Temperate	Summer	Wind	Low	0	800	--
<i>Quercus suber</i> L.	Fagaceae	Fagales	Follonica, Italy	10/05/21- 12/05/21	-18.1 °C / 2 wt%	-19.4 °C	Angiosperm	Temperate	Spring	Wind	Low	0	1000	DT
<i>Sambucus nigra</i> L.	Adoxaceae	Dipsacales	Podivín, Czech Republic	11/05/18- 12/05/18	-12.21 °C / 2 wt%	-14.4 °C	Angiosperm	Temperate	Summer	Animal	Moderate	0	1500	--
<i>Sequoiadendron giganteum</i> (Lindl.) J.Buchholz	Cupressaceae	Cupressales	Leamington Spa	28/02/21	-11.63 °C / 2 wt%	-12.6 °C	Gymnosperm	Temperate	Autumn/ Winter	Wind	High	830	2700	--
<i>Spathiphyllum wallisii</i> Regel	Araceae	Alismateles	House plant	28/11/21	-21.2 °C / 2 wt%	-22.3 °C	Angiosperm	Wet tropical	Year round	Animal	Moderate	1465	1465	--
<i>Taxus baccata</i> L.	Taxaceae	Cupressales	Lednice, Czech Republic	15/03/11- 16/03/11	-18.9 °C / 2 wt%	-20.3 °C	Gymnosperm	Temperate	Spring	Wind	Moderate	0	2200	DT
<i>Triticum aestivum</i> L.	Poaceae	Poales	České Budějovice, Czech Republic	12/06/20	-18.0 °C / 2 wt%	-19.4 °C	Angiosperm	Temperate	Summer	Wind	High	2000	3000	--

* Country indicated if collection location outside the UK

** Pharmallerga® collection, pollen purchased 2020, details lost.

*** Excluded from elevation analysis (artificial hybrid, data not available)

1 Pollen desiccation tolerance

We categorised the pollen of 39 species as desiccation tolerant (DT) or desiccation sensitive (DS), as shown in Table S1, based on information available for specific species, genera, families, or by closely related species (Brewbaker, 1967; Kuznecov, 1970; Bowes, 1990; Hoekstra, 2002; Galgóci et al., 2010; Franchi et al., 2011; Xu et al., 2014; Pacini and Dolferus, 2019). Where this information was not available species were not categorised.

2 All pollen ice nucleation results

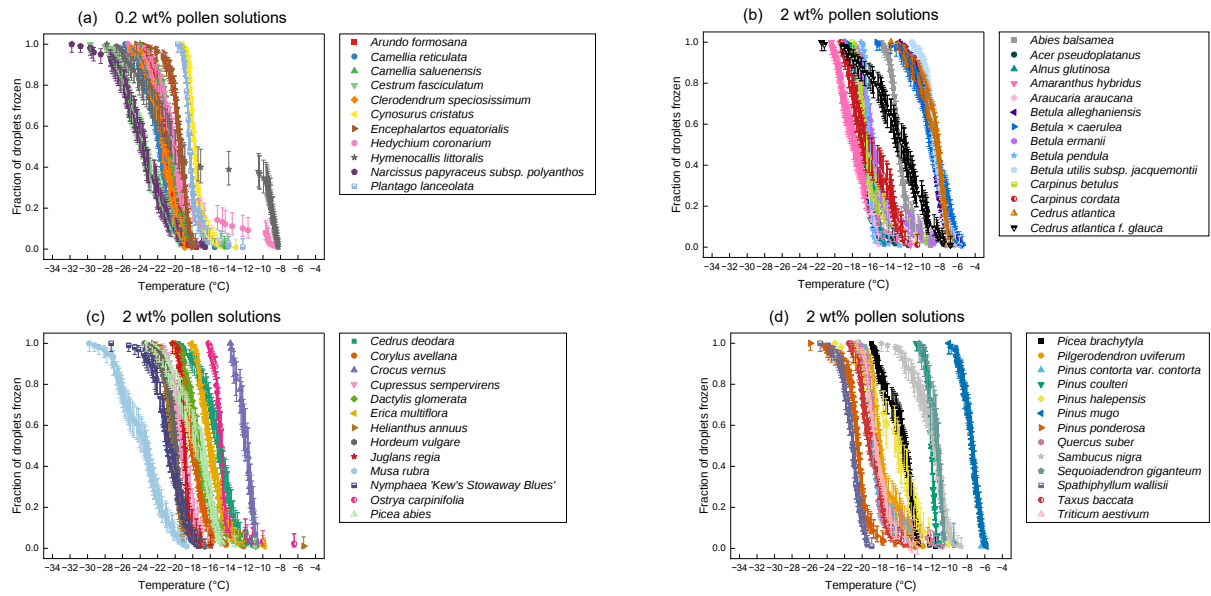
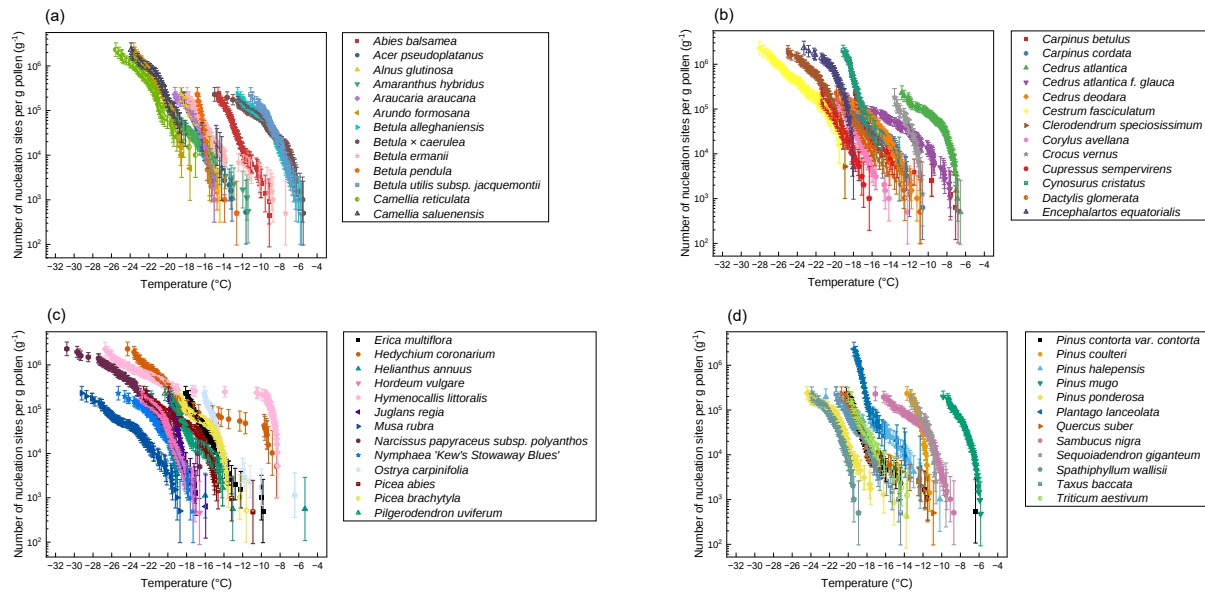
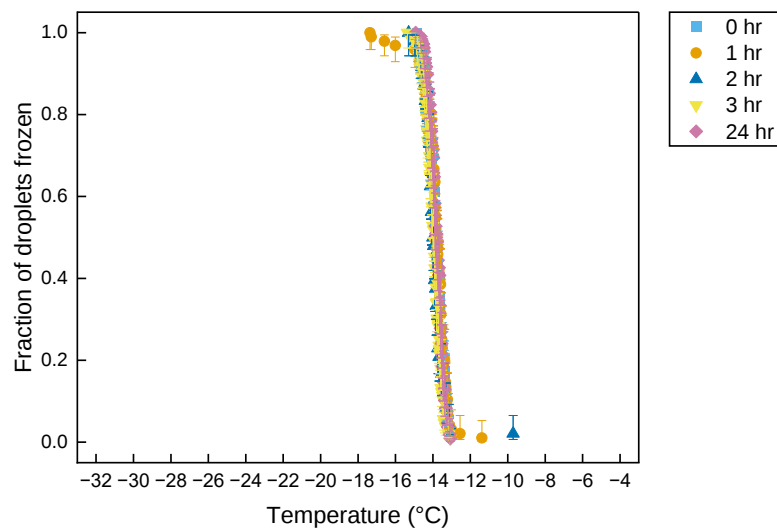


Figure S1: Fraction of droplets frozen plotted against temperature for one microlitre droplets of 51 filtered pollen solutions tested in immersion mode ice nucleation experiments, with (a) showing results for 0.2 wt% pollen solutions and (b), (c), (d) of 2 wt% pollen solutions. 51 filtered pollen solutions tested in immersion mode ice nucleation experiments.



15 **Figure S2: Number of nucleation sites per gram pollen (g^{-1}) plotted against temperature for one microlitre droplets of 51 filtered pollen solutions tested in immersion mode ice nucleation experiments.**

3 Pollen filtration time series results



20 **Figure S3: Fraction of droplets frozen plotted against temperature for one microlitre droplets of *Carpinus betulus* pollen solutions tested in immersion mode ice nucleation experiments, where filtration was carried out at time intervals from 0 hours to 24 hours following preparation of a 20 wt% pollen suspension.**

4 *Pinus mugo* collection results

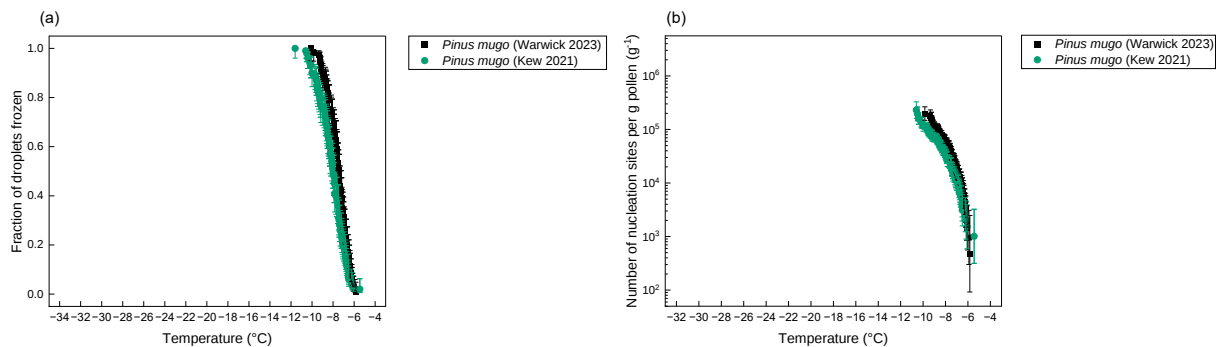


Figure S4: Comparison of ice nucleation activity in collections of *Pinus mugo* pollen from the University of Warwick campus, Coventry (2023) and the Royal Botanic Gardens, Kew, London (2021). (a) Fraction of droplets frozen against temperature for one microlitre droplets of filtered 2 wt% pollen solutions, (b) Number of nucleation sites per gram pollen (g^{-1}) plotted against temperature for one microlitre droplets of filtered 2 wt% pollen solutions.

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