Ice nucleation active bacteria on plants and in precipitation measured using quantitative PCR

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GenBank accession numbers for reference sequences given in Fig. 2 are: *inaZ* (X03035), *inaK* (AF013159), *inaQ* (EU360731), *Ice4* (FN650702), locus tag Psyr1608 (CP000075), *inaV* (AJ001086), *inaZ* interrupted by insert (CP000058), *IceA* (AF387802), locus tag PAJ 3728 (AP012032), *inaA* (X17316), locus tag PANA 0591 (CP001875), locus tag PANA5342_3725 (HE617160), *inaU* (D14992), locus tag PAGR g3607 (CP003085), *iceE* (M26382), locus tag XC 0519 (CP000050), locus tag XCR 4000 (CP002789), *inaX* (X52970), *inaPb* (EU73998) and *inaW* (X04501).

Table S1. Details of sites and samples.

Site	Latitude	Longitude	Alt. (m)	Date sampled	Date snowfall	Sample[1]	Sample wt. (g)	Color of filter	pH	Cond. (µS)
SAREC (Lingle, WY)	42.1290 N	104.394 W	1270	7/15/2010		Alfalfa (Garts 631)	43.0			
″	42.1315 N	104.3965 W	″	″		Corn (Pioneer38&88, RR)	31.5			
″	42.1315 N	104.392 W	″	″		Pinto and Navy beans	38.2			
″	42.1315 N	104.393 W	″	″		Potato (Fl1867)	21.7			
″	42.1290 N	104.393 W	″	″		Sugar beet (Beta 66RR70)	36.4			
″	42.1315 N	104.395 W	″	″		Barley (Burton)	27.4			
″	42.1320 N	104.395 W	″	″		Smooth brome grass	23.4			
″	42.1315 N	104.394 W	″	″		Winter wheat (Jagalene)	45.0			
Laramie Mountains	41.1917 N	105.3204 W	2400	8/5/2010		Crested wheat grass	22.3			
″	″	″	″	″		Smooth brome grass	23.4			
″	″	″	″	″		Western wheatgrass	27.4			
″	41.2385 N	105.3451 W	2434	″		Common timothy	30.5			
″	″	″	″	″		Redtop bentgrass	28.0			
″	41.3864 N	105.4797 W	2370	8/29/2010		Aspen	42.6			
″	41.3880 N	105.4783 W	2398	″		Mountain mahogany	24.9			
″	41.3864 N	105.4797 W	2370	″		Lodgepole pine	42.7			
″	″	″	″	″		Sagebrush	29.3			
Laramie	41.3002 N	105.5770 W	2205	6/10/2010	6/10/2010	Hail (~285 pieces)	122	Very light, hint of color	5.8	-
Laramie Mountains	41.2402 N	105.4407 W	2646	1/26/2011	1/25/2011	Snow (-4 to -6)	1808	Tan	5.7	9.8
Laramie	41.3002 N	105.5770 W	2205	″	″	″	1917	Light tan	6.1	4.6
Laramie Mountains	41.2402 N	105.4407 W	2646	2/1/2011	1/31/2011	Snow (-11 to -24)	1922	Tan	5.6	7.1
Laramie	41.3002 N	105.5770 W	2205	″	″	″	1486	Light tan	5.6	6.0
″	″	″	″	2/6/2011	2/5&6/2011	Snow (-2 to -11)	1832	Light tan	6.0	5.3
″	″	″	″	2/25/2011	2/24&25/2011	Snow (-6 to -9)	1912	Very light	5.6	3.6
Laramie Mountains	41.2402 N	105.4407 W	2646	3/8/2011	3/8/2011	Snow (-3 to -9)	1554	Brown	6.2	8.7
Laramie	41.3002 N	105.5770 W	2205	3/13/2011	3/12&13/2011	Snow (0 to -1)	1208	Very light	5.9	5.2
″	″	″	″	3/29/2011	3/28&29/2011	Snow (-2 to -6)	1189	Very light	5.1	6.4
Laramie	41.3132 N	105.5823 W	2193	1/22/2012	1/22/2012	Snow (-1 to -5)	1401	Brown with black flecks	6.6	4.5
Laramie Mountains	41.2402 N	105.4407 W	2646	2/3/2012	2/2&3/2012	Snow (-4 to -7)	1190	Light brown	7.0	6.9
Laramie	41.3002 N	105.5770 W	2205	″	″	″	1368	Chocolate brown, fine organics	6.1	19.4
Laramie Mountains	41.2258 N	105.4447 W	2676	2/13/2012	2/12&13/2012	Snow (-2 to -6)	1661	Light tan	5.3	3.9
Laramie site 1	41.3002 N	105.5770 W	2205	2/28/2012	2/28/2012	Snow (-3 to -6)	1164	Very light	6.6	2.4
Laramie site 2	41.3064 N	105.5732 W	2193	″	″	″	1066	Light brown	6.3	5.4
Laramie Mountains	41.2258 N	105.4447 W	2676	4/16/2012	4/15&16/2012	Snow (+3 to -4)	1699	Fine grey brown	6.3	6.0

[1] For snow samples, the ground air temperature range in Laramie (°C) during the snowfall is given in parentheses.

Table S2. Relative efficiencies of qPCR amplification of isolates compared to reference strain *Ps. syringae* Cit7. Threshold signal levels used to derive the comparisons are shown in Fig. S1. All reactions were initiated with 10 ng of genomic DNA. FP, false positive; No A, no amplification; MP, mis-prime.

		Amplification efficiency of isolates, relative to *Ps. syringae* Cit7, using primer pairs 3308f/3463r and 3341fb/3462r1 (×)			
Isolate	Species	3308f/ 3463r		Notes	3341fb/ 3462r1
Cit7	*Ps. syringae*	1			1
BXIN4	*Ps. syringae*/*congelans*	2.4			1.2
GCh5Fc	*Ps. syringae*/*congelans*	6.4			2.0
BXIN3	*Ps. syringae*/*congelans*	0.6			6.6
PCa2bi	*Ps. viridiflava*	2.0		MP off non-*ina* gene by both primers (292 bp, 86% similarity to a potassium channel protein in *Ps. syringae*)	► FP
Sco1009b	*Ps. syringae* pv. *atropurpurea*	No A			0.3
GrF	*Pa. ananatis*	2.1		Minor MP off non-*ina* gene (373 bp, 99% sequence similarity to a maltose import ATP-binding protein in *Pa. ananatis*)	► 0.9
SBPci	*Pa. agglomerans*	0.05	◄	Underestimation mainly by MP off base 3356[1], generating a 146 bp amplicon	0.7
Sba1007a	*X. campestris* pv. *campestris*	No A			0.5
Sba1007bi	*X. campestris* pv. *campestris*	No A			0.4
Sbr1009a	*X. translucens*	0.005	◄	Underestimation by MP off base 3356[1], generating a 146 bp amplicon	0.13
MU26	*Ps. fluorescens*	0.3			5.5
LSb	*Ps.* sp. (*putida*?)	1.5		Multiple MPs and cause not clear, but not mis-match to forward primer	► FP
MM3b	*Ps.* sp. (*putida*?)	0.6		Overestimation by efficient MP off base 3317[1], generating a 186 bp amplicon	► 4.2
BF81Fb	*Ps. koreensis*/*fluorescens*/ *putida*	0.2	◄	Underestimation by MP off base 3356[1], generating an additional 146 bp amplicon	3.8
χ16	*Ps. auricularis*	0.5			1.4
χ17	*Ps. poae*	0.03	◄	Underestimation possibly by exclusion of primer by secondary fold. No mis-matches with either primer	1.2
GraPa8	*Ps.* sp./*auricularis*	0.5			1.9

[1] Using the base numbering according to that of *inaZ*

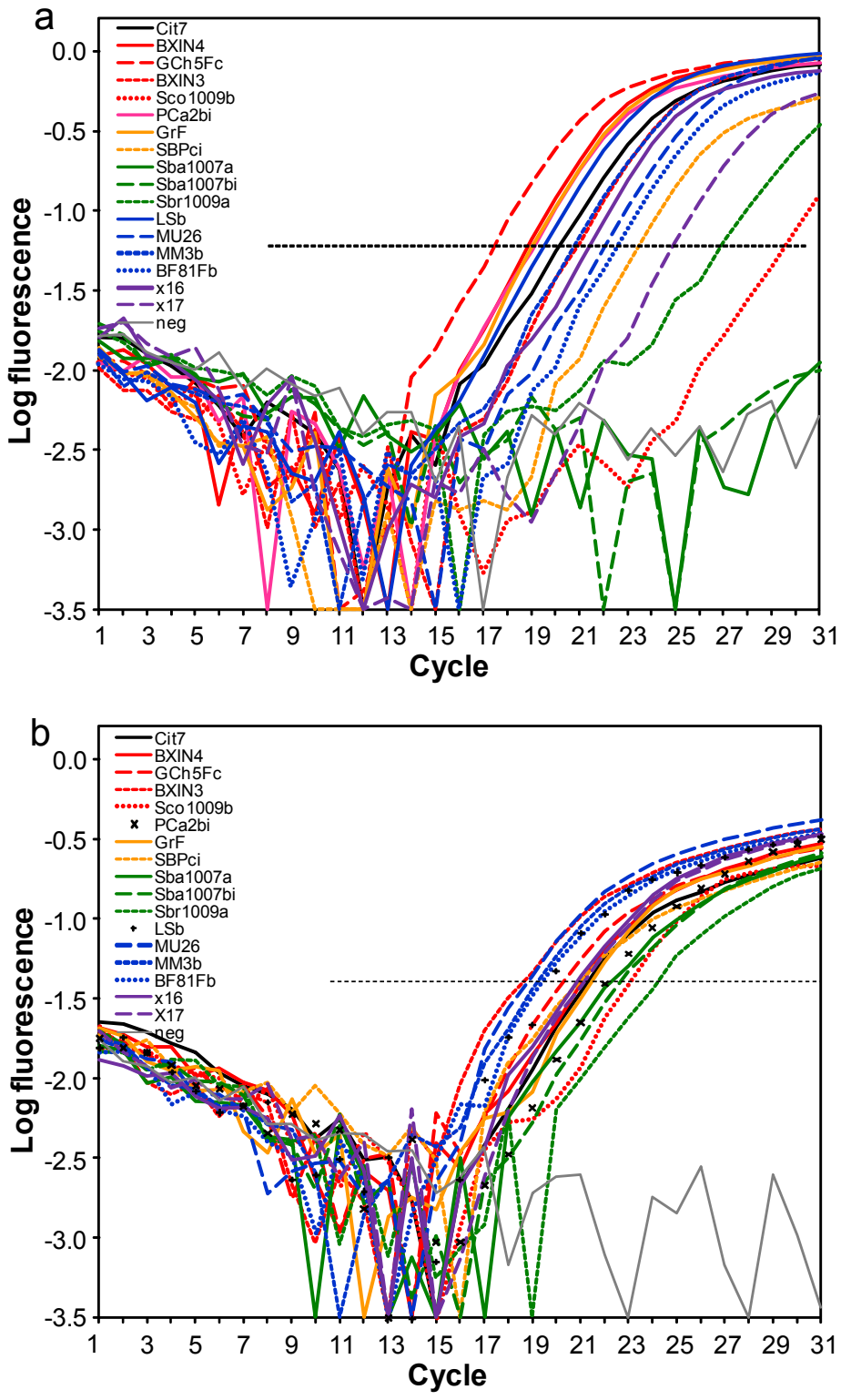

Figure S1. Comparison of qPCR performance for primer pairs 3308f/3463r (a) and 3341fb/3462r1 (b) designed to amplify a broad range of *ina* gene alleles. Horizontal dashed lines are the threshold signal values used to compare amplification efficiencies in Table S2.

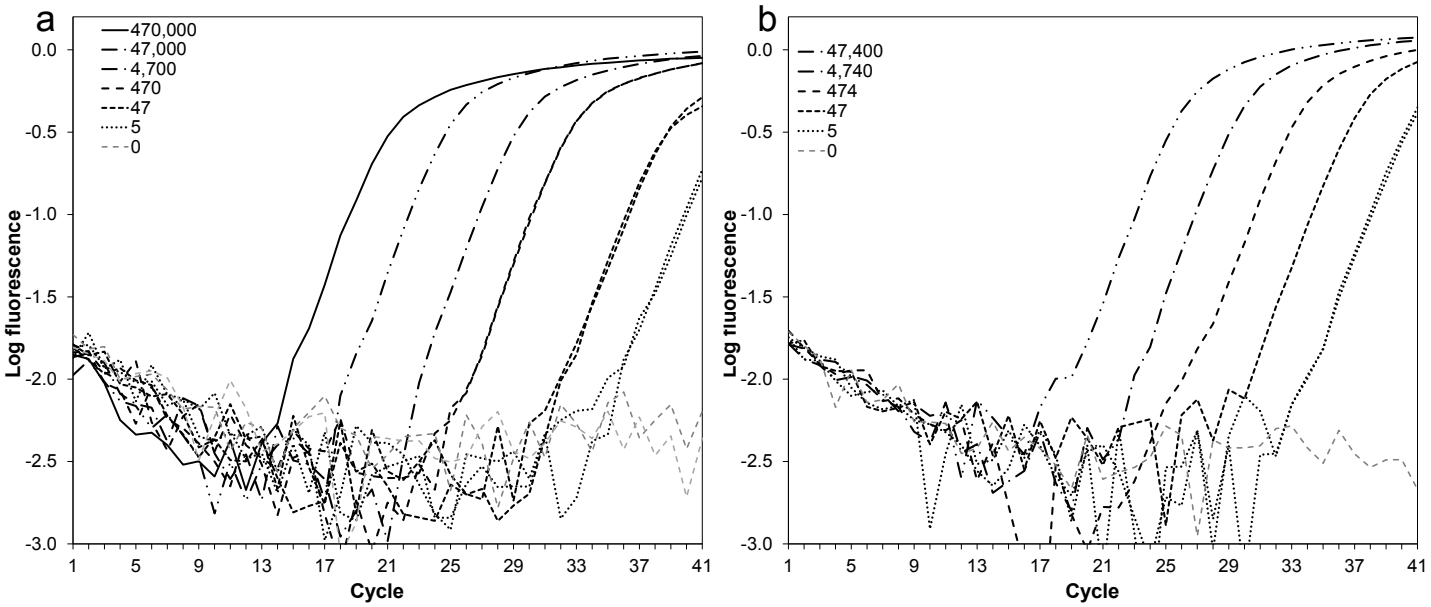

Figure S2. (a) Quantitative PCR standard series using primers 3308f/3462r1 with *Ps. syringae* Cit7 DNA (annealing and extension at 53°C, 1% DMSO). The legend values are the number of *ina* gene copies per reaction. Standards were made by extraction of DNA from 50 µl aliquots of cells taken from a dilution series of the isolate. When reverse primer 3463r was used the result was identical except for a lack of amplification of the lowest 5 gene copy standard. (b) qPCR using 3380f with 3463r but under more stringent PCR conditions (annealing and extension at 54°C, 4% DMSO), enabling 5 gene copies to be amplified.

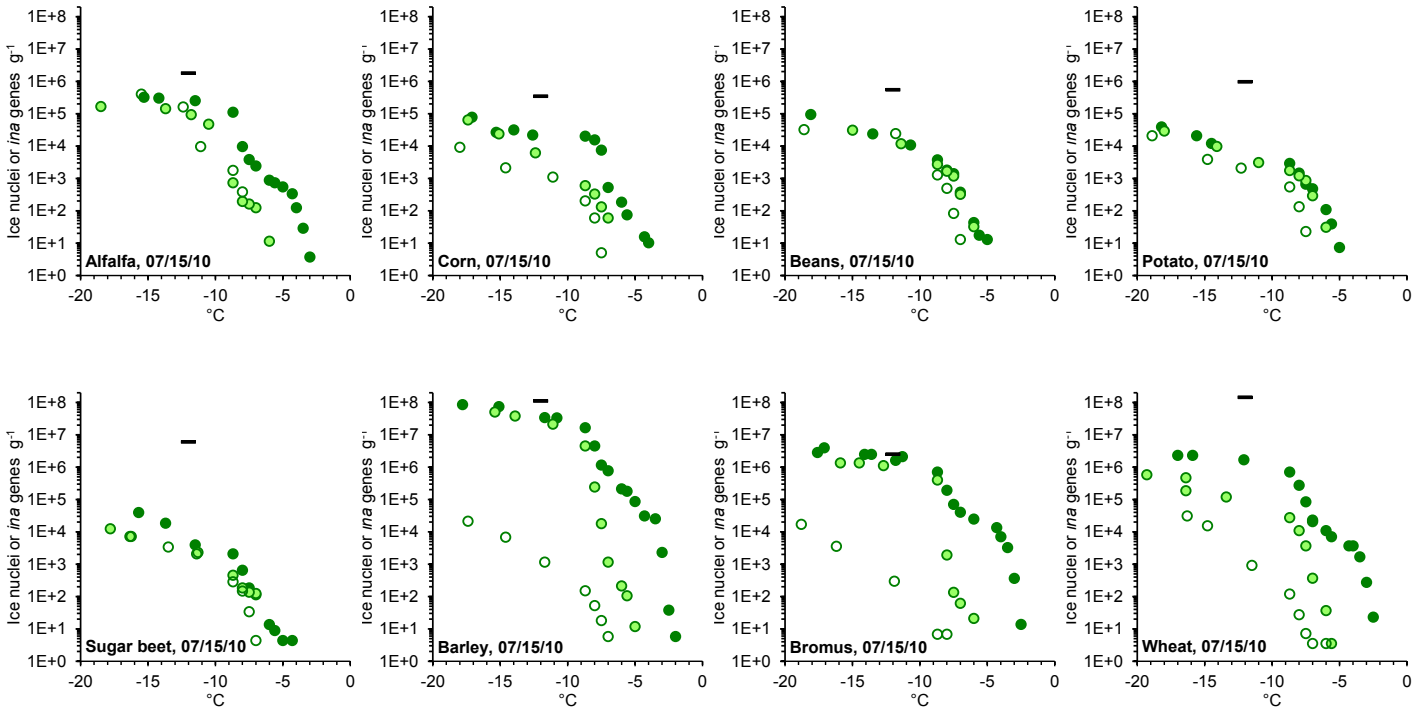

Figure S3. Ice nucleating particle temperature spectra in leaf washings from irrigated crops at SAREC. Solid circles are unamended samples, shaded circles are samples heated to 60°C and open circles are samples heated to 105°C. *ina* gene copy concentrations are shown as a bar at -12°C.

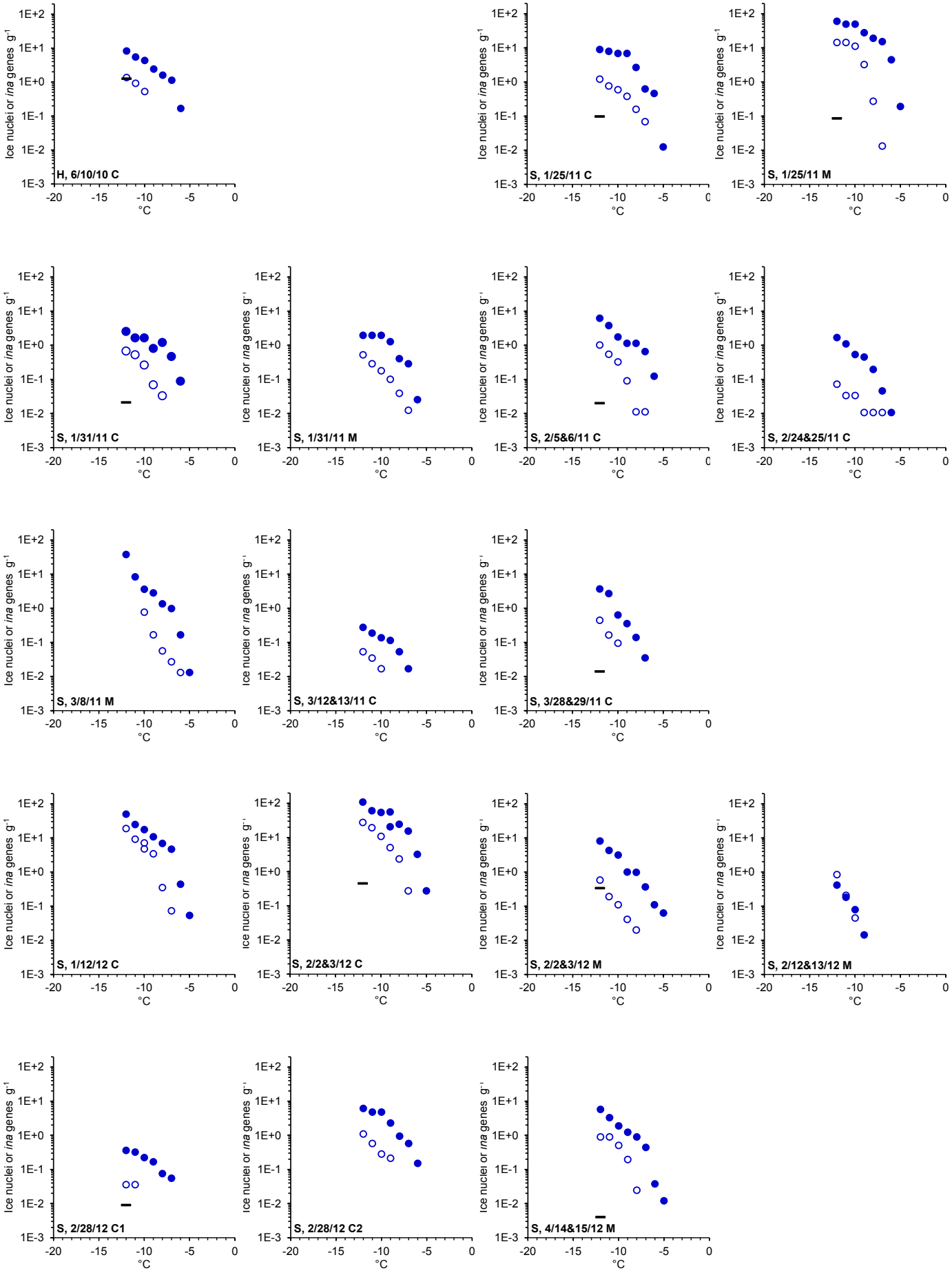
Ice nuclei or ina genes g-1
1E+2
1E+1
1E+0
1E-1
1E-2
1E-3
-20
-15
-10
-5
0
°C
H, 6/10/10 C
S, 1/25/11 C
S, 1/25/11 M
S, 1/31/11 C
S, 1/31/11 M
S, 2/5&6/11 C
S, 2/24&25/11 C
S, 3/8/11 M
S, 3/12&13/11 C
S, 3/28&29/11 C
S, 1/12/12 C
S, 2/2&3/12 C
S, 2/2&3/12 M
S, 2/12&13/12 M
S, 2/28/12 C1
S, 2/28/12 C2
S, 4/14&15/12 M

Figure S4. Ice nucleating particle temperature spectra in precipitation samples. Solid circles are unamended samples and open circles are samples heated to 105°C. *ina* gene copy concentrations, when detected, are shown as a bar at -12°C. H and S indicate hail and snow samples, and C and M indicate samples collected in Laramie city and in the Laramie Mountains, respectively.