

SUPPLEMENTAL TABLES AND FIGURES

P. Bello and K.J. Bradford. 2016. Single-seed oxygen consumption measurements and population-based threshold models link respiration and germination rates under diverse conditions. Seed Science Research.

Supplemental Table S1. Germination and respiration parameters for seeds and treatment conditions.

Variety	Experiment Conditions/Treatments		Germination Parameters		POD Curve Values						ASTEC Values - Oxygen Depletion - Median Curve						
			R50 Curve		R75 Curve		R25 Curve										
			(h)	%	(h)	%	(h)	%	(h)	%	%/h	(h)	%/h	(h)	(h)		
Tomato	Temperature	Temperature	T50	Final	R50(50)	Final	R75(50)	Final	R25(50)	Final	SMR	IMT	OMR	RGT	R50	AUC50	
		15	130.90	93%	126.00	98%	82.71	98%	153.00	98%	0.29%	104.07	0.92%	180.26	126.46	101.97	
		20	75.06	97%	87.63	100%	58.13	100%	110.50	100%	0.42%	65.96	1.05%	135.17	88.34	70.81	
	High Water Potential	25	61.17	98%	67.07	100%	46.75	100%	80.01	100%	0.49%	53.23	2.01%	93.25	67.78	54.86	
		Water Potential	T50	Final	R50(50)	Final	R75(50)	Final	R25(50)	Final	SMR	IMT	OMR	RGT	R50	AUC50	
		0	61.17	98%	67.07	100%	46.75	100%	80.01	100%	0.49%	53.23	2.01%	93.25	67.78	54.86	
	Low Water Potential	-0.25	65.53	94%	84.67	100%	51.36	100%	119.92	100%	0.46%	51.89	0.88%	147.28	85.42	66.87	
		-0.4	97.10	84%	103.97	100%	54.52	100%	156.85	98%	0.47%	61.13	0.57%	193.47	104.98	79.85	
		-1	-	0%	151.00	83%	75.75	100%	-	11%	0.33%	-	0.33%	299.67	152.84	115.16	
	Temperature and High Water Potential	-1.5	-	0%	-	45%	93.25	98%	-	6%	0.26%	-	0.26%	388.85	-	-	
		-2	-	0%	-	28%	102.25	98%	-	6%	0.23%	-	0.23%	440.53	-	-	
		Temperature	Water Potential	T50	Final	R50(50)	Final	R75(50)	Final	R25(50)	Final	SMR	IMT	OMR	RGT	R50	AUC50
		15	0	130.90	93%	126.00	98%	82.71	98%	153.00	98%	0.29%	104.07	0.92%	180.26	126.46	101.97
		15	-0.25	156.98	85%	170.50	100%	104.07	100%	211.10	99%	0.25%	151.40	0.70%	240.77	170.86	135.33
		20	0	76.38	97%	87.63	100%	58.13	100%	110.50	100%	0.42%	65.96	1.05%	135.17	88.34	70.81
		20	-0.25	83.97	100%	101.50	100%	58.67	100%	160.00	100%	0.37%	43.30	0.58%	188.33	102.02	79.55
		20	-0.4	129.96	95%	124.67	100%	71.33	100%	213.50	93%	0.32%	56.51	0.48%	228.53	125.46	96.97
		25	0	61.17	98%	67.07	100%	46.75	100%	80.01	100%	0.49%	53.23	2.01%	93.25	67.78	54.86
		25	-0.25	65.53	94%	84.67	100%	51.36	100%	119.92	100%	0.46%	51.89	0.88%	147.28	85.42	66.87
	Temperature and Low Water Potential	25	-0.4	97.10	84%	103.97	100%	54.52	100%	156.85	98%	0.47%	61.13	0.57%	193.47	104.98	79.85
		15	-1	0.00	0%	-	0%	119.50	100%	-	0%	0.20%	-	0.20%	493.90	-	-
		15	-1.5	0.00	0%	-	0%	167.25	86%	-	0%	0.14%	-	0.14%	700.80	-	-
		15	-2	0.00	0%	-	0%	-	41%	-	0%	0.11%	-	0.11%	927.16	-	-
		20	-1	0.00	0%	173.50	76%	78.00	100%	-	0%	0.29%	-	0.29%	347.39	179.19	132.55
		20	-1.5	0.00	0%	-	15%	121.50	98%	-	0%	0.22%	-	0.22%	455.60	-	-
		20	-2	0.00	0%	-	2%	130.50	96%	-	0%	0.20%	-	0.20%	515.73	-	-
		25	-1	0.00	0%	151.00	83%	75.75	100%	-	11%	0.33%	-	0.33%	299.67	152.84	115.16
		25	-1.5	0.00	0%	-	45%	93.25	98%	-	6%	0.26%	-	0.26%	388.85	-	-
		25	-2	0.00	0%	-	28%	102.25	98%	-	6%	0.23%	-	0.23%	440.53	-	-
	Respiratory Inhibitors KCN and SHAM	KCN mM	SHAM mM	T50	Final	R50(50)	Final	R75(50)	Final	R25(50)	Final	SMR	IMT	OMR	RGT	R50	AUC50
		0	0	55.00	98%	60.75	100%	52.13	100%	66.50	100%	0.38%	53.96	4.03%	73.75	61.45	53.57
		0.1	0	76.50	96%	86.50	100%	72.50	100%	93.00	100%	0.33%	80.12	3.65%	100.28	86.95	74.23
		0.2	0	194.96	98%	152.00	100%	90.50	100%	163.25	98%	0.31%	151.68	2.61%	173.19	153.50	121.60
		0.3	0	269.46	65%	231.00	85%	108.25	100%	253.00	72%	0.21%	243.08	2.20%	265.09	232.53	174.76
		0	0.5	58.98	100%	75.75	100%	65.88	100%	81.83	100%	0.30%	67.84	3.71%	89.45	76.50	67.08
		0	1	79.33	100%	86.50	100%	72.83	100%	93.50	100%	0.31%	78.45	3.24%	102.00	86.86	74.90
		0	2	108.33	100%	120.00	100%	83.00	100%	127.00	96%	0.31%	116.26	3.55%	134.66	120.39	99.16
		0	3	129.69	70%	142.00	94%	80.67	100%	151.50	88%	0.30%	140.62	2.57%	162.96	143.21	112.50
		0.1	0.3	76.30	100%	87.25	100%	70.00	100%	93.75	100%	0.37%	81.03	3.48%	101.75	87.75	74.43
		0.1	1	87.03	100%	94.00	100%	74.67	100%	101.00	100%	0.33%	88.51	3.32%	109.89	95.37	80.19
		0.1	3	131.74	100%	150.00	85%	84.00	98%	161.00	75%	0.29%	151.35	3.26%	168.86	151.93	118.73
		0.3	3	-	-	-	14%	142.46	100%	-	0%	0.19%	122.54	0.10%	881.21	-	-
		0.3	5	-	-	-	2%	160.79	100%	-	0%	0.18%	154.06	0.08%	1114.76	-	-
		Hormones - ABA	ABA μM	T50	Final	R50(50)	Final	R75(50)	Final	R25(50)	Final	SMR	IMT	OMR	RGT	R50	AUC50
	0		72.00	92%	93.00	92%	79.33	96%	98.80	92%	0.24%	77.71	2.38%	112.12	93.36	80.91	
	1		240.00	79%	220.00	79%	108.00	96%	254.00	75%	0.20%	234.37	1.29%	276.05	237.29	179.46	
	3.3		471.27	46%	-	46%	112.00	100%	-	46%	0.22%	165.13	0.05%	1462.23	-	-	
	10		-	8%	-	17%	116.00	96%	-	8%	0.24%	121.51	0.05%	1568.13	-	-	
	33		-	0%	-	0%	137.00	96%	-	0%	0.23%	91.93	0.08%	1052.83	-	-	
	Hormones - GA	GA μM	T50	Final	R50(50)	Final	R75(50)	Final	R25(50)	Final	SMR	IMT	OMR	RGT	R50	AUC50	
		0	-	0%	-	0%	-	0%	-	0%	0.11%	87.08	0.01%	9596.79	-	-	
		0.1	-	0%	-	0%	-	0%	-	0%	0.10%	82.57	0.01%	8957.17	-	-	
		1	-	4%	-	4%	-	4%	-	4%	0.11%	81.06	0.01%	8234.23	-	-	
		10	864.00	25%	-	32%	-	36%	-	32%	0.14%	106.16	0.01%	7761.46	-	-	
		100	116.00	88%	139.00	91%	122.00	95%	146.00	91%	0.17%	127.86	2.78%	156.11	139.48	121.19	
	Lettuce	Accelerated Aging	Aging days	T50	Final	R50(50)	Final	R75(50)	Final	R25(50)	Final	SMR	IMT	OMR	RGT	R50	AUC50
			Primed (-1 days)	10.12	100.0%	25	0.99	17.00	100%	30.50	0.95	1.01%	17.00	4.08%	37.49	26.36	21.61
			0	11.52	100.0%	30.5	1.00	18.67	100%	39.00	0.98	1.09%	20.96	2.83%	48.63	31.29	25.07
			2	16.73	100.0%	35.5	1.00	22.83	100%	44.50	0.91	1.03%	26.45	2.67%	54.26	35.81	29.25
			4	23.60	100.0%	44.5	0.93	26.43	100%	54.00	0.77	1.02%	39.01	2.32%	65.72	44.53	35.49
			6	36.00	93.8%	59	0.77	36.25	92%	67.00	0.53	0.82%	61.80	4.38%	73.37	59.58	46.92
	Radish	Controlled Deterioration	Aging days	T50	Final	R50(50)	Final	R75(50)	Final	R25(50)	Final	SMR	IMT	OMR	RGT	R50	AUC50
			0	11.71	100%	16.94	100%	12.85	100%	19.81	100%	1.92%	13.77	7.71%	23.55	17.21	14.45
			45	21.89	96%	24.50	100%	15.33	100%	29.00	100%	1.70%	22.73	5.45%	34.19	24.85	20.10
			66	32.07	88%	31.17	96%	15.58	96%	36.63	96%	1.53%	30.34	4.22%	43.20	31.17	23.99
			80	44.67	83%	36.50	91%	16.88	96%	50.50	91%	1.37%	48.29	3.44%	58.23	37.07	27.80

ASTEC values - oxygen depletion median curve: Starting Metabolism Rate (SMR), Increasing Metabolism Time (IMT), Oxygen Metabolism Rate (OMR), Relative Germination Time (RGT), Time to 50% oxygen depletion (R50) and Area Under the curve at 50% oxygen level (AUC50). See Bradford et al. (2013) for further details.

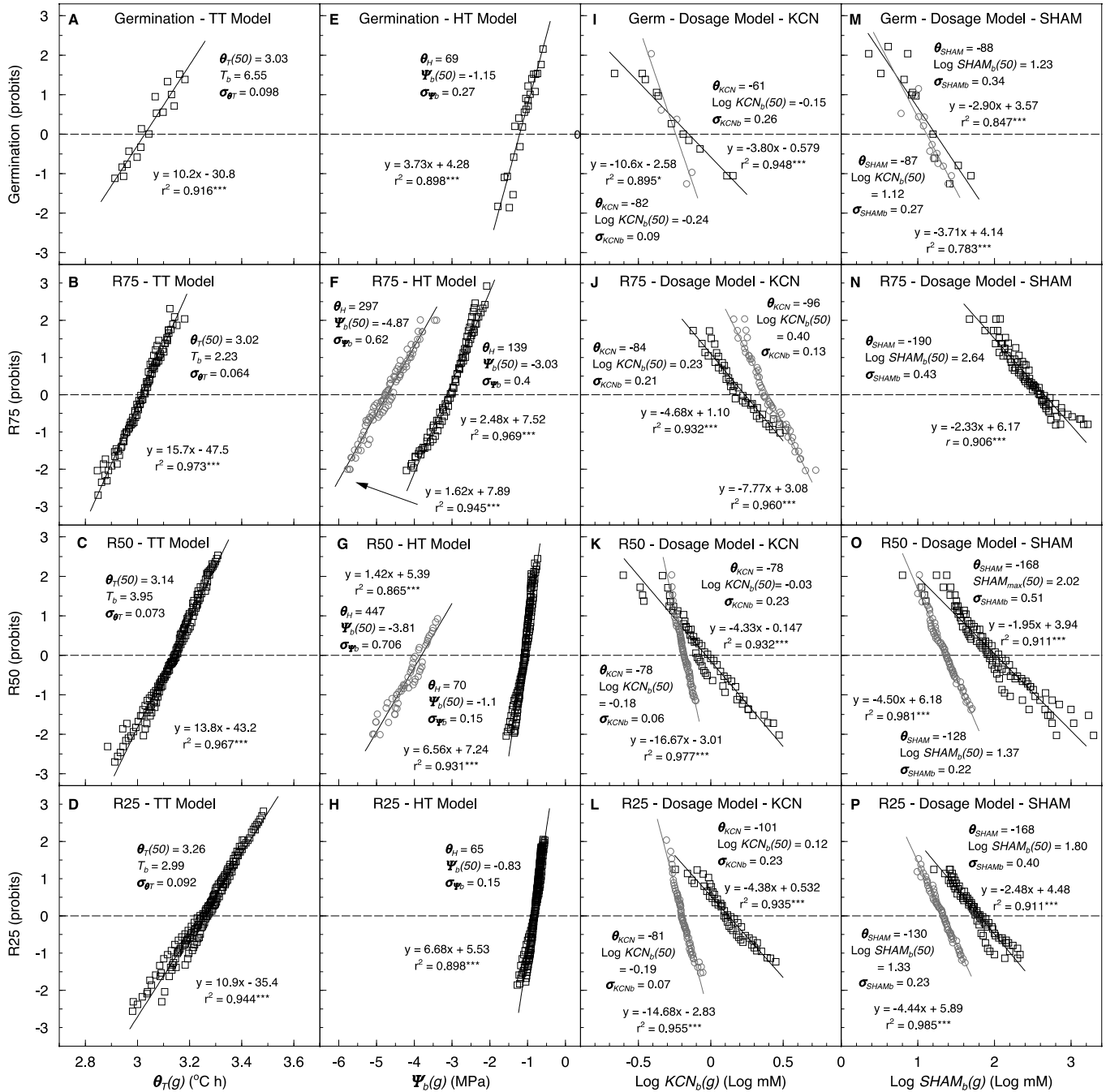


Figure S1. Probit regressions to fit the thermal time (TT), hydrothermal time (HT) and inhibitor (Dosage) models to data for germination and respiration of tomato seeds. The data are from the time courses in Figure 3 and additional data for SHAM. The model parameter values shown are: $\theta_T(50)$ = median thermal time (°C h); T_b = base temperature (°C); σ_{θ_T} = standard deviation of θ_T values (°C h); θ_H = hydrotime constant (MPa h); $\Psi_b(50)$ = median base water potential (MPa); σ_{Ψ_b} = standard deviation of Ψ_b values; θ_{KCN} = KCN dosage time constant (log mM h); $KCN_b(50)$ = median KCN concentration at which germination or respiration would be completely prevented (log mM); σ_{KCNb} = standard deviation of $KCN_b(g)$ values (log mM), θ_{SHAM} = SHAM dosage time constant (log mM h); $SHAM_b(50)$ = median SHAM dosage at which germination or respiration would be completely prevented (log mM); σ_{SHAMB} = standard deviation of $SHAM_b(g)$ values (log mM) (see also Table 1). In cases where a second model calculation was needed (panels F, G, I-M, O and P), data are also displayed as gray circles.

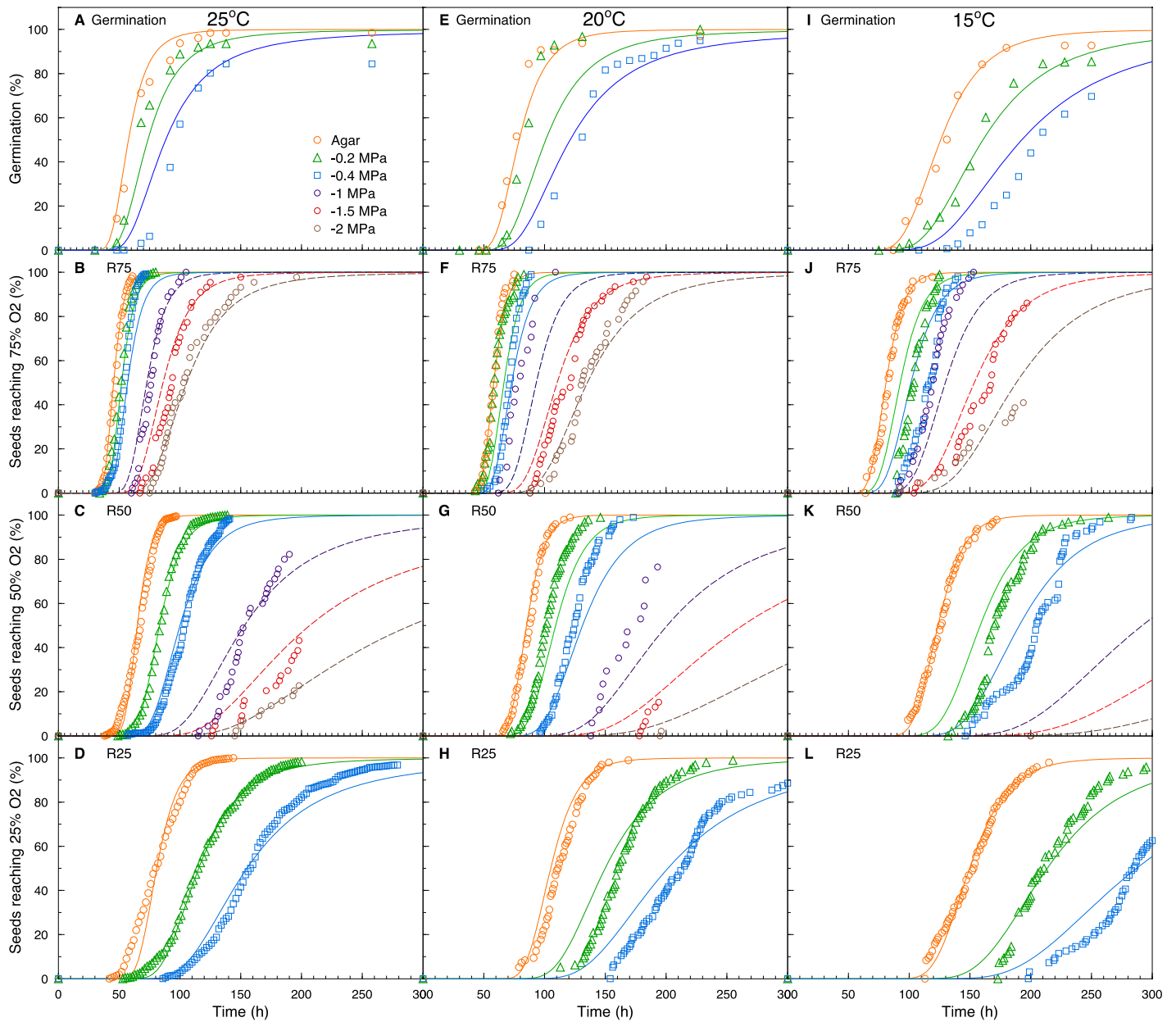


Figure S2. Germination time courses (A, E, I) and R75, R50 and R25 POD time courses (B-D, F-H, and J-L) for tomato seeds imbibed at a range of Ψ values (see legend in panel A) at 25 (A-D), 20 (E-H) or 15°C (I-L). The time courses predicted by the hydrothermal time model are represented as solid (high Ψ range) or dashed (low Ψ range) lines for the respective conditions.

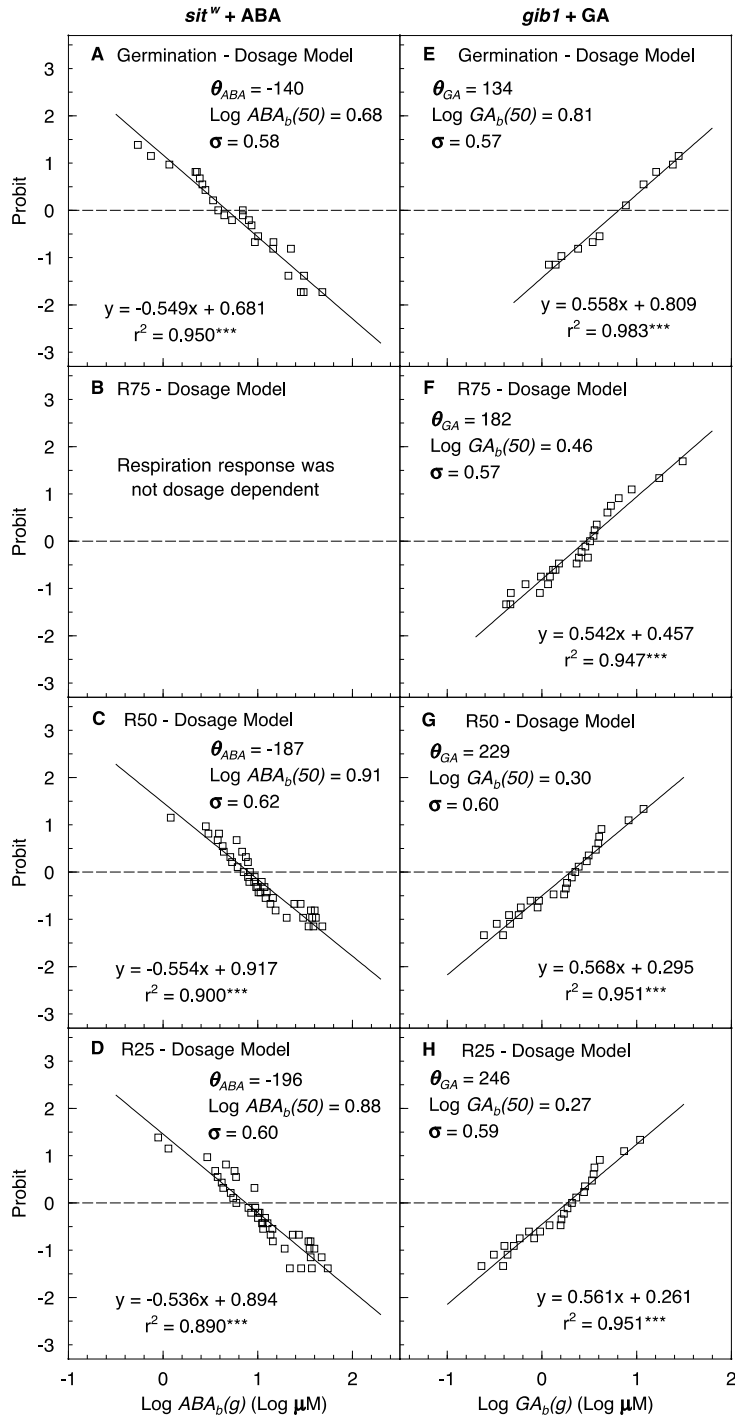


Figure S3. Probit regressions to fit the hormone (dosage) model to data for germination and respiration of tomato seeds in response to ABA and GA. The data are from the time courses in Figure 6. The model parameter values shown are: θ_{ABA} = ABA-time constant (log $\mu\text{M h}$); $ABA_b(50)$ = median base ABA concentration that would inhibit germination or respiration of 50% of the seeds (log μM); σ_{ABA_b} = standard deviation of ABA_b values; θ_{GA} = GA-time constant (log $\mu\text{M h}$); $GA_b(50)$ = median base GA concentration that would stimulate germination of 50% of the seeds (log μM); σ_{GA_b} = standard deviation of GA_b values (see also Table 1). Values are not shown for R75 for the ABA dosage model, as all concentrations of ABA caused a reduction in respiration rates, but there was little effect of ABA concentration *per se* (see Fig. 6B). Thus, the respiration pattern for this early phase of imbibition was not consistent with the assumptions of the model.

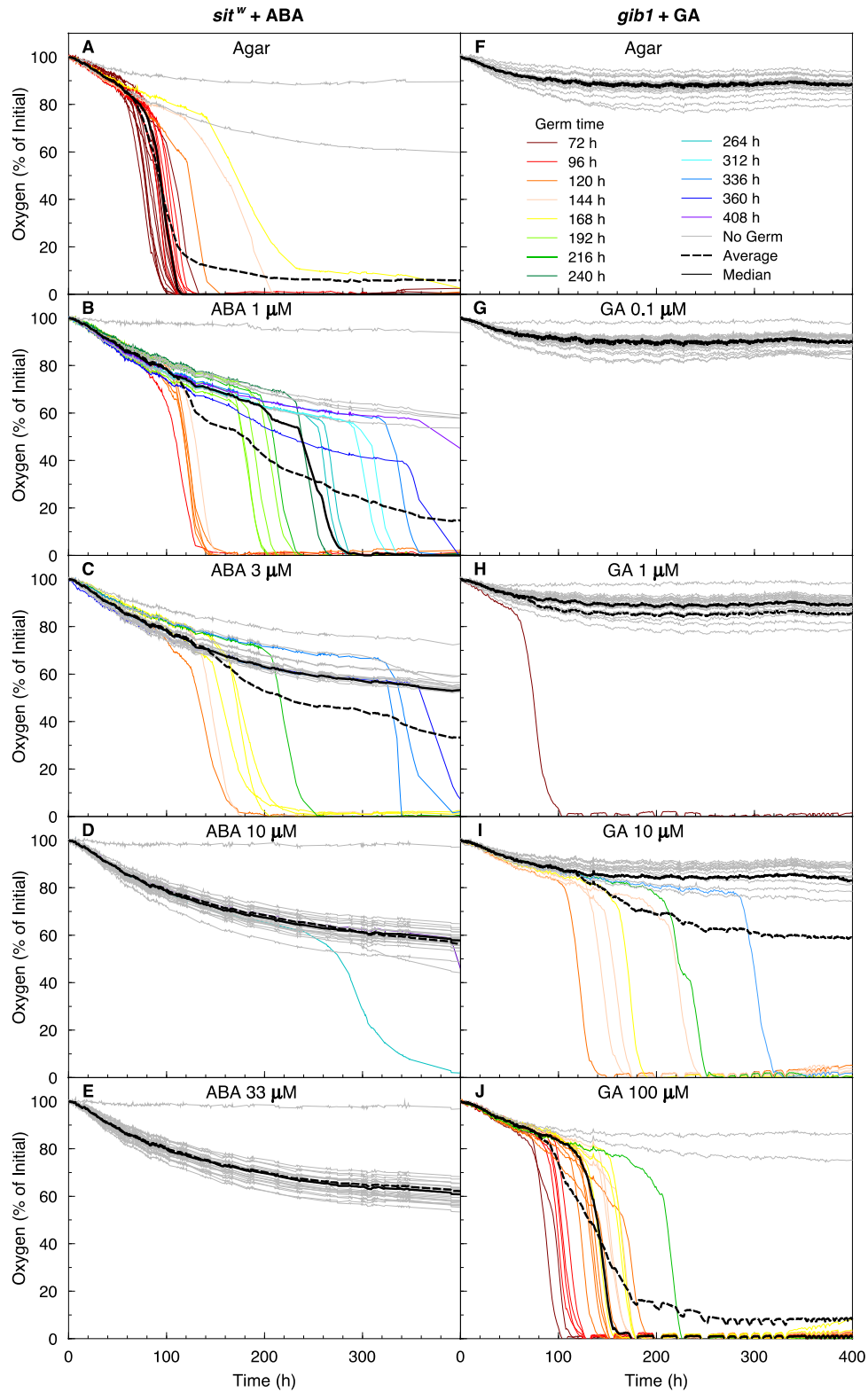


Figure S4. Q2 oxygen depletion curves of individual seeds and average and median curves for *sit^w* seeds plus ABA (A-E) or *gib1* seeds plus GA (F-J). The time courses are colored to reflect the times by which radicle emergence had occurred for that seed (see legend in panel F). As germination was delayed (ABA) or advanced (GA) by increasing concentrations of the hormones, the respiratory patterns were affected similarly. Only seeds that transitioned to a more rapid oxygen consumption rate germinated, and the time of radicle emergence was closely associated with the time of that transition.

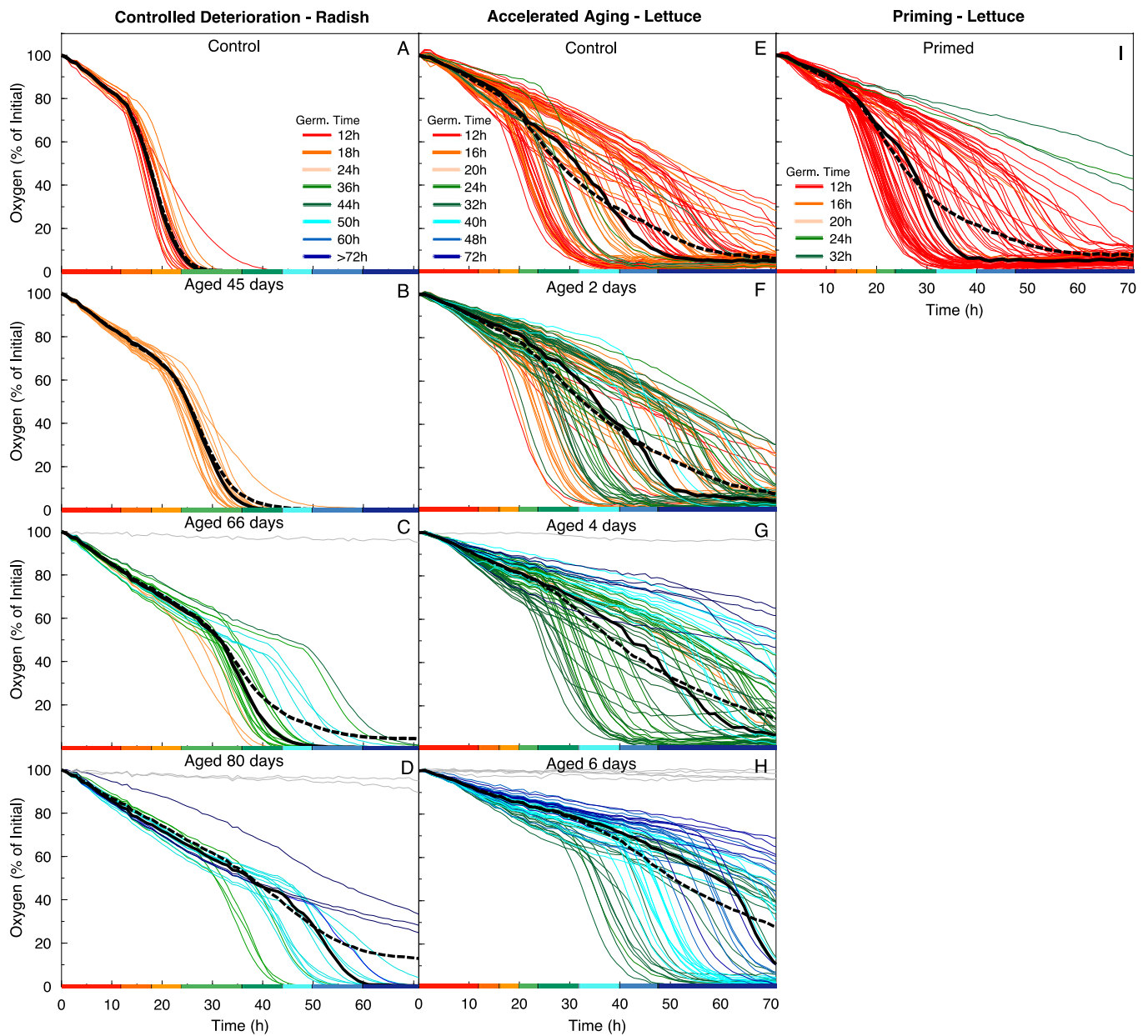


Figure S5. Oxygen consumption time courses of individual untreated (control) radish seeds (**A**) that had been subjected to 45 (**B**), 66 (**C**), 80 (**D**) days of controlled deterioration at 33% RH and 50°C. Similar curves are shown for individual lettuce seeds that were untreated (**E**) or had been subjected to 2 (**F**), 4 (**G**), or 6 (**H**) days of controlled deterioration at 75% RH and 50°C. Control lettuce seeds were also subjected to priming (prehydration and drying) prior to measurement (**I**). Each curve indicates the oxygen consumption time course of an individual seed, and is color coded to reflect the time by which radicle emergence had occurred for that seed (see legends in panels A, E and I and also the colored bars on the x-axes). Additional grey curves represent non-germinating seeds. Averages (dashed curves) and medians (solid lines) for the seed populations are shown in black.

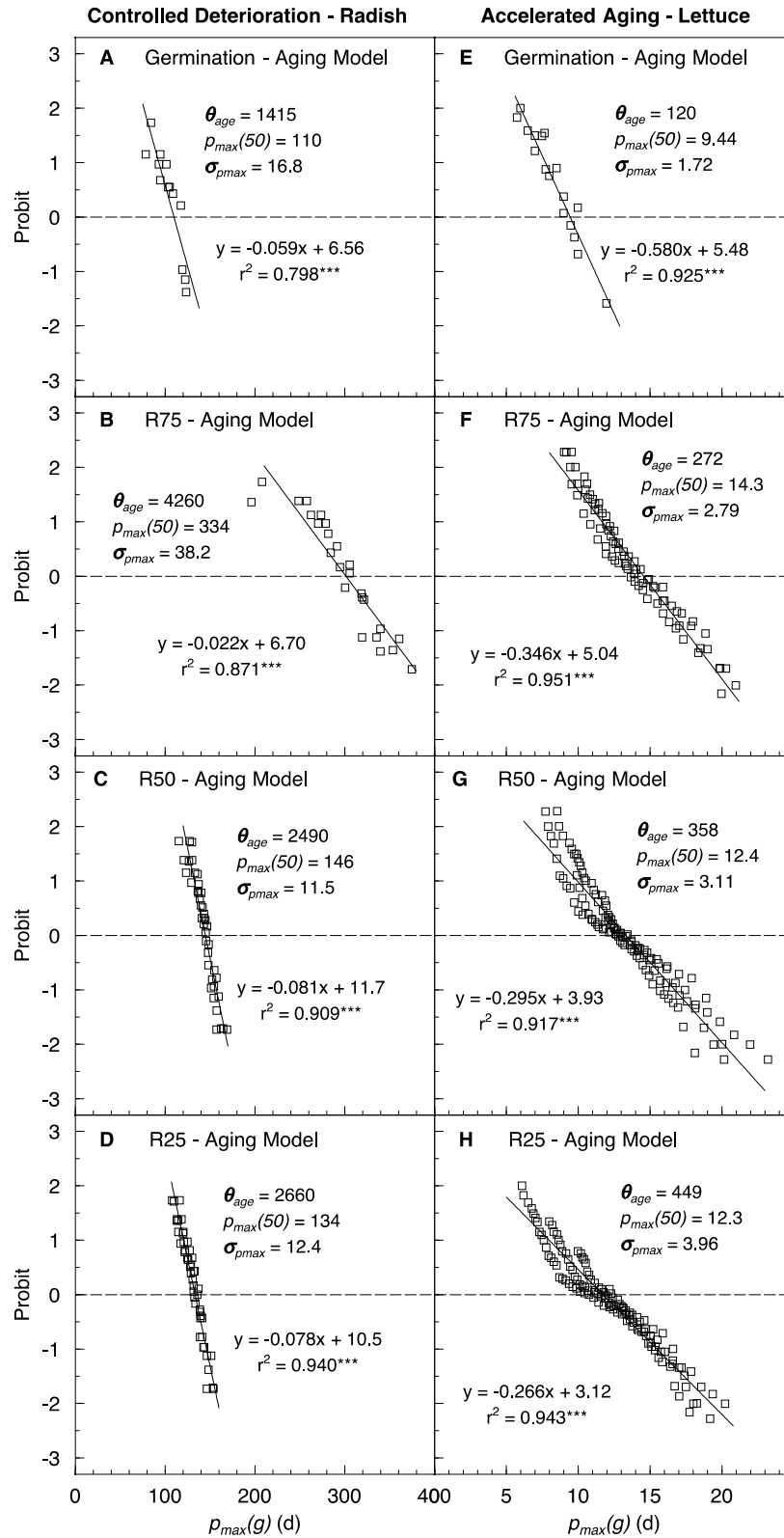


Figure S6. Probit regressions to fit the aging model to data for germination and respiration of radish (**A-D**) and lettuce (**E-H**) seeds. The data are from the time courses in Figure 7. The model parameter values shown are: θ_{age} = aging time constant (aging days h); $p_{max}(50)$ = median days of aging at which germination or respiration would be completely prevented (days); σ_{pmax} = standard deviation of p_{max} values (days) (see also Table 1).

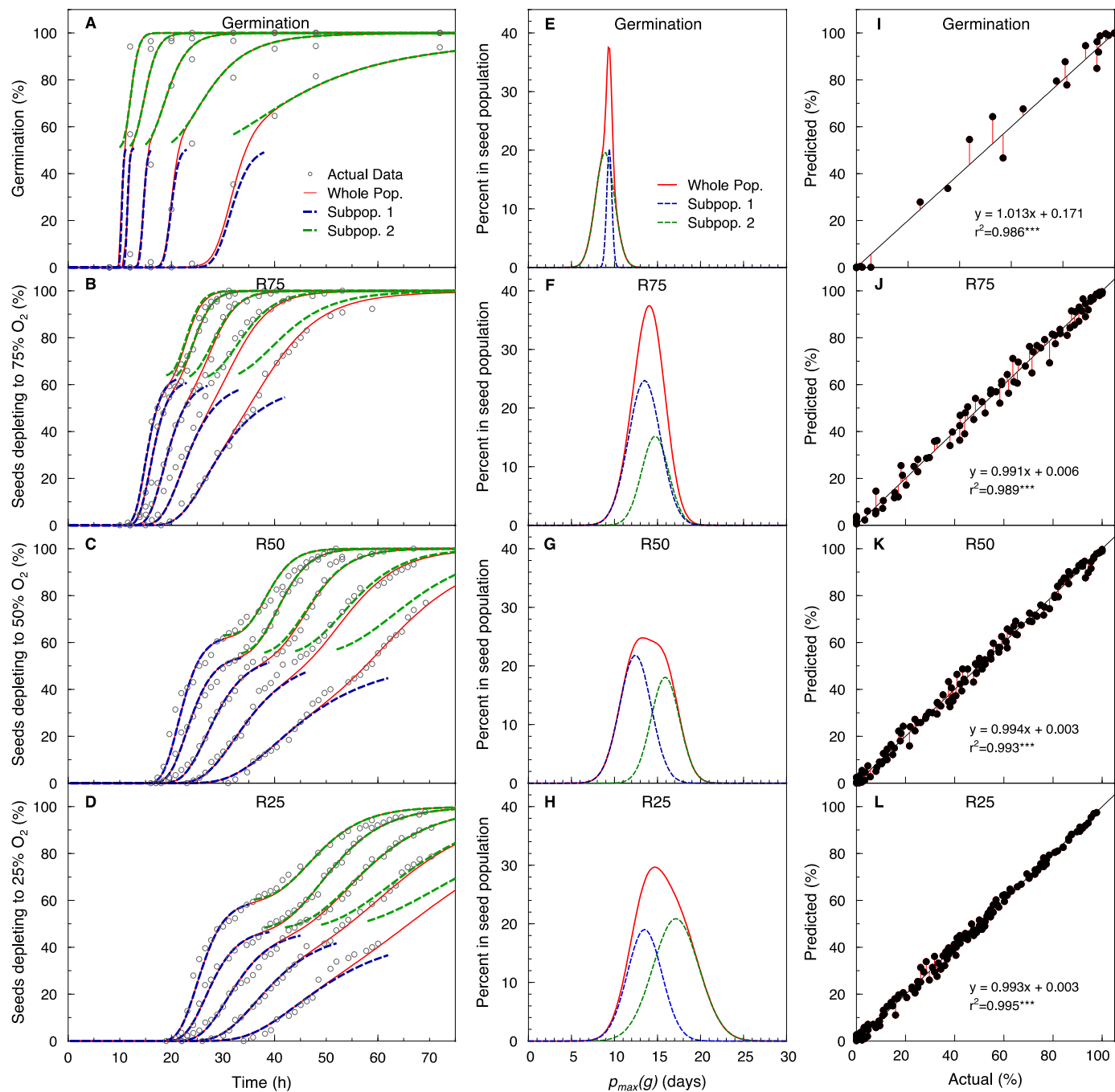


Figure S7. Development of subpopulation models for primed and aged lettuce seeds. The data (gray circles in panels A-D) exhibited clear evidence of subpopulations that were responding differently to priming and aging (data from Fig. 7E-H). Assuming that the seed lot was composed of two distinct subpopulations, we calculated two different models and respective parameters for different fractions of the population (panels A-D, purple and green dashed lines). Overall values obtained by summing the predicted time courses of the two subpopulations closely matched the overall respiration time courses (panels A-D, solid red lines; see also Fig. 7I-L). The normal distributions of the two subpopulations are shown based on the medians and standard deviations of the aging model (panels E-H; Table S2) that provided the best fit to the data (data for primed seeds were not used in fitting the model) (panels I-L). The summed distribution of the entire population is also shown (panels E-H, solid red lines). Subpopulation models were optimized using Excel Solver to minimize the sum of squared

residuals (SSR) between actual germination/respiration and predicted values (panels I-L, red lines, solid black lines and black circles, respectively) by solving for all model parameters based on the following equation:

$$T(gr) = f_1 \{ [p - (\theta_{age1} / t_{gr1}) - p_{max}(50)_1] / \sigma_{pmax1} \} + (1 - f_1) \{ [p - (\theta_{age2} / t_{gr2}) - p_{max}(50)_2] / \sigma_{pmax2} \}$$

where $T(gr)$ is the distribution of germination or respiration (e.g., R75, R50 or R25) times of the total population, (f_i) is the fraction of the total population in subpopulation 1, p is the aging period, θ_{age1} and θ_{age2} are the aging time constants for subpopulations 1 and 2, t_{gr1} and t_{gr2} are the germination or respiration times of individual seeds in subpopulations 1 and 2, $p_{max}(50)_1$ and $p_{max}(50)_2$ are the medians of the distributions of p_{max} values for subpopulations 1 and 2, and σ_{pmax1} and σ_{pmax2} are the standard deviations of p_{max} values among seeds in subpopulations 1 and 2. Thus, the value of f_1 and the parameters of both subpopulation models were optimized by minimizing the deviation of the total population model from the actual data (minimizing SSR; panels I-L) (Table S2). The curves in panels A-D illustrate the two populations by superimposing their predicted time courses on the same axis and having each population reach a maximum at its fraction of the total population (dashed blue and green lines). The figures illustrate that at longer aging times, the RXX times of seeds in the two populations overlap, resulting in loss of the distinct double-sigmoid shape that is evident in primed and control seeds (e.g., panel C). Although data for primed seeds were not included in calculations of the subpopulation equation described above, using the same model parameters with a “negative ageing” period ($p = -1$ day) and increasing the fraction of seeds in the faster subpopulation (f_1) enabled the model to match the data well. Interestingly, even though germination was observed too infrequently to detect subpopulations directly in the data, applying the same subpopulation approach as was used for the POD curves also resulted in an improved fit of the model to the germination time course data (panels A, I). Theoretically, additional subpopulation terms could be added to the equation above (i.e., $f_3 = 1 - f_1 - f_2$, etc.) if justified by the data.

Table S2. Parameters for the subpopulation models for respiration of primed and aged lettuce seeds shown in Figure S7. A value of -1 aging days was used in the model for the primed seeds.

Germination / O ₂ depletion	Subpopulation	Fraction (%)	θ_{age} (day h)	$p_{max(50)}$ (days)	σ_{pmax} (days)	SSR	r^2
Germination	1	50.7	108	9.4	0.3	0.060	0.986***
	2	49.3	134	8.9	1.1		
R75	1	62.0	223	13.5	1.9	0.099	0.989***
	2	38.0	365	14.7	1.6		
R50	1	54.7	296	12.4	1.8	0.078	0.993***
	2	45.3	657	15.9	1.7		
R25	1	47.7	374	13.5	2.0	0.050	0.995***
	2	52.3	880	17.1	2.67		