Three-dimensional Models Represent Seed Moisture Content as a Function of Relative Humidity and Temperature

Jian Fang and Frank Moore

Department of Horticulture and Landscape Architecture, Colorado State University, Fort Collins, CO 80523

Eric Roos¹ and Christina Walters

U.S. Department of Agriculture, Agricultural Research Service, National Seed Storage Laboratory, 1111 South Mason Street, Fort Collins, CO 80521-4500

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Abstract. Seed moisture content (MC) has been considered the most important factor controlling physiological reactions in seeds, and MC changes with relative humidity (RH) and temperature (T). This relationship is revealed by studying the interaction of RH and T at equilibrium. Cucumber (*Cucumis sativus* L.), lettuce (*Lactuca sativa* L.), maize (*Zea mays* L.), onion (*Allium cepa* L.), pea (*Pisum sativum* L.), and watermelon (*Citrullus lanatus* M. & N.) seeds were equilibrated over sulfuric acid (1% RH) and various saturated salt solutions (5.5% to 93% RH) at temperatures from 5 to 50 °C. Best-fit subset models were selected from the complete third-order model $MC = \beta_0 + \beta_1 \cdot RH + \beta_2 \cdot T + \beta_3 \cdot RH^2 + \beta_4 \cdot T^2 + \beta_5 \cdot RH \cdot T + \beta_6 \cdot RH^3 + \beta_7 \cdot T^3 + \beta_8 \cdot RH \cdot T^2 + \beta_9 \cdot RH^2 \cdot T$, using Mallows' minimum Cp as the selection criterion. All six best subset models (R^2 , 0.98 to 0.99) had the same functional form, $MC = \beta_0 + \beta_1 \cdot RH + \beta_2 \cdot T + \beta_3 \cdot RH^2 + \beta_5 \cdot RH \cdot T + \beta_6 \cdot RH^3 + \beta_9 \cdot RH^2 \cdot T$. Coefficients had essentially the same respective values among all species except onion and pea, for which some coefficients were statistically different from those of the other species ($P \leq 0.05$). All models indicated that seed MC increased as RH increased and decreased as T increased; but RH had the greater influence. The inverse relationship between seed MC and T, although slight, was evident in the response surfaces. The interaction effect of RH and T on MC was significant at $P \leq 0.001$. These results suggest that orthodox seed species respond similarly to T and RH. This in turn suggests that a common model could be developed and used for optimizing seed storage environments.

Equilibrium relationships between moisture content (MC) and relative humidity (RH) are revealed by moisture sorption isotherms (Vertucci and Roos, 1990, 1993). Construction of such isotherms is a convenient method for assaying the physical state of water in relatively dry tissues (Rupley et al., 1983; Vertucci and Leopold, 1987). Changes in the physical status of water are associated with changes in the physiological activities of seeds (Leopold and Vertucci, 1989; Vertucci and Leopold, 1986; Vertucci and Roos, 1990).

Achieving the optimum seed MC for long-term storage is a goal of gene banks, such as the National Seed Storage Laboratory. Understanding the interaction of temperature (T) and RH on seed MC, and how this varies

among species, is an important step in designing storage facilities.

Numerous two-dimensional presentations or response curves have been proposed to describe the relationships between RH and MC (D'Arcy and Watt, 1970; Yang and Cenkowski, 1995). However, a three-dimensional model that includes a T term is needed to generate a response surface to analyze interaction. Our purpose was to investigate the interactive effect of RH and T on MC using best-fit models to generate three-dimensional response surfaces.

Seeds of cucumber ('Straight Eight', Gurney's Seed & Nursery Co., Yankton, S.D.; initial germination, 91%), lettuce ('Black-Seeded Simpson', Gurney's Seed & Nursery Co.; initial germination, 94%), maize (Hybrid, 'Pioneer 3541', lot 88-10, Pioneer Hi-Bred International Inc., Des Moines, Iowa; initial germination, 95%), onion ('Yellow Sweet Spanish', Henry Field Seed & Nursery Co., Shenandoah, Iowa; initial germination, 93%), pea ('Extra-Early Alaska', Gurney's Seed & Nursery Co.; initial germination, 92%), and watermelon ('Sugar Baby', Henry Field Seed & Nursery Co.; initial germination, 91%) were equilibrated over H_2SO_4 (>99.5% concentration, 1% RH) and 11 saturated salt solutions (5.5% to 93% RH) at temperatures from 5 to 50 °C (Table 1). At weekly intervals, two replicates of 10 seeds or 1 gm of seeds each from each of 60 treatments/species were assayed for MC. The experiment continued until a constant moisture content at each RH/T combination was reached, which required from 30 to 45 d.

To determine the MC, seeds were weighed prior to drying at 105 °C for 24 h. Seed moisture content was expressed on a dry-weight basis.

Since equilibrium moisture content of seeds is controlled by two factors, T and RH, interaction of these two factors was examined by comparing surfaces generated by best-fit models and also by analyzing the models. Models for each species were selected from a complete third-order model $MC = \beta_0 + \beta_1 \cdot RH + \beta_2 \cdot T + \beta_3 \cdot RH^2 + \beta_4 \cdot T^2 + \beta_5 \cdot RH \cdot T + \beta_6 \cdot RH^3 + \beta_7 \cdot T^3 + \beta_8 \cdot RH \cdot T^2 + \beta_9 \cdot RH^2 \cdot T$ using Mallows' minimum Cp as a criterion and Mallows' graphical method (Draper and Smith, 1981) to evaluate bias and random error. Subset models (511) were examined for each species by the program (Kleinbaum et al., 1988). A factorial experiment permitted comparison of main and interaction effects on seed MC.

Results and Discussion

Figure 1A is presented to illustrate goodness of fit. Response surfaces generated by the best subset models for moisture content in cucumber, lettuce, maize, onion, pea, and wa-

Table 1. Relative humidities (%) of various saturated salt solutions and sulfuric acid at different temperatures (Vertucci and Roos, 1993).

Salt	Temp (°C)				
	5	15	25	35	50
H_2SO_4 (>99.5%)	1	1	1	1	1
ZnCl ₂	5.5	5.5	5.5	5.5	5.5
NaOH	9	8	7	7	6
LiCl	15	14	13	11.5	11
CaBr ₂	23	22	16.5	15	14
MgCl ₂	33.5	33	32.5	32.5	31.5
K ₂ CO ₃	43	43	43	41.5	41
Mg(NO ₃) ₂	56	54.5	53	50.5	46
NH ₄ NO ₃	72	68	62	62.5	61
NaCl	75.5	75.5	75	75	74.5
KCl	88	86	85	84	80.5
KNO ₃	93	92.5	91	90	85

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¹To whom reprint requests should be addressed.

Fig. 1. (A) Seed moisture content data clouds where each point is the mean of two observations. (B) Response surfaces for each of six species, generated by using the best-fit seed moisture content (MC) models having the form $MC = \beta_0 + \beta_1 \cdot RH + \beta_2 \cdot T + \beta_3 \cdot RH^2 + \beta_4 \cdot RH \cdot T + \beta_5 \cdot RH^3 + \beta_6 \cdot RH^2 \cdot T$. RH = relative humidity; T = temperature.

Table 2. Coefficients of the six models predicting the influence of temperature (T) and relative humidity (RH) on seed moisture content (MC). $MC = \beta_0 + \beta_1 \cdot RH + \beta_2 \cdot T + \beta_3 \cdot RH^2 + \beta_4 \cdot RH \cdot T + \beta_5 \cdot RH^3 + \beta_6 \cdot RH^2 \cdot T$, was the best fit subset model for all six species.

Species	R^2	β_0	β_1	β_2	β_3	β_4	β_5	β_6
Cucumber	0.98	3.2 a [*]	0.22 a	-0.072 a	-0.0048 a	0.0014 a	0.000049 a	-0.000019 a
Lettuce	0.99	3.4 a	0.22 a	-0.076 a	-0.0052 a	0.0020 a	0.000054 ab	-0.000023 a
Maize	0.99	4.0 a	0.31 ab	-0.089 a	-0.0065 a	0.0012 a	0.000061 ab	-0.000014 a
Onion	0.99	3.7 a	0.44 b	-0.095 a	-0.0100 bc	0.0017 a	0.000090 ab	-0.000022 a
Pea	0.98	4.7 a	0.42 ab	-0.120 a	-0.0120 c	0.0040 a	0.000120 b	-0.000052 a
Watermelon	0.98	3.7 a	0.30 ab	-0.089 a	-0.0067 ab	0.0020 a	0.000063 ab	-0.000025 a

^{*}Mean separation within columns by 95% confidence limits.

Table 3. Mean squares for main effects and interaction of relative humidity (RH), and temperature (T), on seed moisture content of six species. All 18 sources of variation, considering main, interaction, and species, were significant at $P \leq 0.001$.

Source of variation	df	Cucumber	Lettuce	Maize	Onion	Pea	Watermelon
RH	4	55	52	80	102	146	73
T	11	258	276	402	494	780	314
RH $\times$ T	44	1.7	1.5	1.1	2.0	7.7	1.7
Residual	60	0.007	0.007	0.010	0.008	0.011	0.012

termelon are presented in Fig. 1B. The six models having the lowest Cp value and least bias had the same functional form, $MC = \beta_0 + \beta_1 \cdot RH + \beta_2 \cdot T + \beta_3 \cdot RH^2 + \beta_4 \cdot RH \cdot T + \beta_5 \cdot RH^3 + \beta_6 \cdot RH^2 \cdot T$. Coefficients $\beta_0, \beta_2, \beta_3, \beta_6$ were the same ($P \leq 0.05$) for all species. Specifically, β_1 onion was different from cucumber and lettuce; β_3 onion and pea was different from cucumber, lettuce, and maize; β_4 pea was different from watermelon; and β_6 pea was different from cucumber (Table 2). Most parameter values among species were essentially the same (Table 2); however, onion and pea β_3 values were larger by an order of magnitude than those of the other four species. Likewise, the highest MCs estimated by the pea and onion models ranged from 34% to 45%, and 30% to 36%, respectively, vs. 20% to 30% estimated by the models of the other four species. The generally higher MC estimated by the pea model was attributed to the relatively low lipid content of pea (Vertucci and Roos, 1990). The effect of seed composition in determining seed MC is reflected in the models objectively selected and supported in this study.

Notable was the fact that interaction terms $RH \cdot T$ and $RH^2 \cdot T$ were included in the final model, suggesting that interaction was significant. Furthermore, this was supported by the results of the factorial experiment that compared main and interaction effects over species. These results also suggest that both factors had a greater effect on the MC of pea and onion seeds than on cucumber, lettuce, maize, and watermelon seeds (Table 3).

Coefficients of multiple determination were significant at $P \leq 0.01$ and ranged from 0.98 to 0.99. Mallows' graphical method (Draper and

Smith, 1981) for determining bias indicated that only the cucumber seed MC model exhibited a degree of bias (data not shown), and perhaps would not be quite as good a predictor as the other five species. The standardized coefficients (Table 4) support the response surfaces in Fig. 1B, in that RH has a strong positive effect on MC but T has a slightly negative effect.

Conclusions

Models for cucumber, lettuce, maize, onion, pea, and watermelon seeds had the same functional form. Based on the models and model-generated response surfaces, RH has a greater influence on seed MC than does T. Relative humidity has a strong positive effect while T has a slightly negative effect on MC. The extremely strong interactive effect of RH and T on seed MC is documented by these results. Also, one might justify fitting the Cp selected seven-parameter form to a combined species data set, $n = 6 \times 60$ (six species, 12 relative humidities, and five temperatures), thus providing a general model for the purpose of interpolation of the interaction of RH and T on orthodox seed MC.

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Table 4. Standardized² regression coefficients β_1 and β_2 associated with linear effects of relative humidity (RH) and temperature (T) variables, respectively.

Species	RH	T
Cucumber	1.36	-0.22
Lettuce	1.31	-0.23
Maize	1.53	-0.22
Onion	1.94	-0.21
Pea	1.46	-0.22
Watermelon	1.65	-0.25

²Software (Dixon, 1981) computes coefficients from a standardized revision of the model where $\beta_j' = \beta_j(S_j/S_y)$ $j = 1, 2, \dots, k$ (Kleinbaum et al., 1988). Column RH is significantly different from column T at $P \leq 0.05$ based on a t test, and variances are equal based on an equal variance test (Fox et al., 1995).

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