Soaking Temperature of Dried Tuberous Roots Influences Hydration Kinetics and Growth of *Ranunculus asiaticus* (L.)

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Abstract. The published literature is inconsistent with recommendations for hydrating *Ranunculus asiaticus* (L.) dried tuberous roots, a common practice in commercial production systems for this ornamental geophyte. Imbibition rate increased with hydration temperature but to lower equilibrium moisture content than when hydrated at cooler temperatures. In the greenhouse, survival was predicted to be greatest when tubers were hydrated at 20 °C. Plant height, visual quality, and foliar dry weight followed a similar trend 4 weeks after planting. These results demonstrate that a hydration temperature between 15 and 25 °C is required to obtain good quality when growing *R. asiaticus* from its dried tuberous roots.

*Ranunculus asiaticus* is a traditional cut flower and flowering potted plant that has become popular in early spring gardening and landscape designs (Hamrick, 2003). *R. asiaticus* is often grown from seed or from its dried tuberous roots (hereafter abbreviated “roots”), which flow faster and more profusely than from seed (Meynet, 1993). The roots of *R. asiaticus* are well adapted to lengthy dry storage and have therefore been identified as “resurrection geophytes” (Beruto et al., 2009; Kamenetsky et al., 2005). It is common commercial practice to hydrate the dry roots before planting, because this provides handling uniformity and facilitates fungicide application (Y. Liberman, personal communication); however, the published information on hydration duration and temperature is inconsistent. Meynet (1993) suggested direct planting without a hydration treatment, whereas others recommended soaking the roots in slowly running water for 24 h before planting (De Hertogh, 1996; Umie! and Hagildai, 1999); specific hydration temperature recommendations were not provided. While investigating the effect of stratification on *R. asiaticus* flowering, Ohkawa (1986) submerged the roots 8 h at 6 °C before planting. It is important to establish a unified *R. asiaticus* root hydration protocol for future scientific work and for consistent commercial production.

Because several processes could limit the rate of hydration in *R. asiaticus* roots, an appropriate hydration kinetics model must be selected. An empirical model for describing moisture sorption curves, the Peleg model, has been used to model water uptake in a number of dehydrated and rehydrated products including seeds of kidney bean (*Phaseolus vulgaris* L.), chick pea (*Cicer arietinum* L.), and field pea (*Pisum sativum* L.) along with rice (*Oryza sativa* L.) and other cereal grains and other food products (Abu-Ghannam and McKenna, 1997; Bello et al., 2008; Hung et al., 1993; Peleg, 1988; Prasad et al., 2010; Sarchetti et al., 2003; Sopade et al., 1992; Turhan et al., 2002). The advantage of using Peleg’s model for estimating moisture uptake is the ability to predict long-range moisture gains from relatively short duration experiments (Peleg, 1988).

In this research, the Peleg model was investigated for applicability in modeling *R. asiaticus* hydration. Subsequent greenhouse experiments demonstrate the influence of hydration temperature on plant growth.

Materials and Methods

Hydration kinetics. Dried roots of *Ranunculus asiaticus* (‘Tecolote Pink’) were obtained from a commercial producer (see specific experiment) and randomly assigned to one of three distilled water temperature regimes: 5, 20, or 35 °C. There were five replications per treatment with five sub-sample roots per replicate. Roots were submerged for 1 h, removed, blotted dry, weighed, and then re-submerged. Data were collected hourly for 12 h and then after at 24 h and 30 h. After 30 h, roots were placed in a 70 °C oven and dried until constant weight was achieved.

Model fitting. Turhan et al. (2002) used the Peleg model to describe moisture sorption in chickpea; much of their work is adapted for interpretation and presentation of our results. The two-parameter sorption equation proposed by Peleg (1988) is considered for modeling *R. asiaticus* hydration:

$$ M(t) = M_0 \pm \frac{t}{K_1 + K_2 t} $$

where *M(t)* is the dry basis moisture content of roots at time *t* (%), *M₀* is the initial moisture content (%), *K₁* is the Peleg rate constant (h⁻¹), and *K₂* is the Peleg capacity constant (%). Because we are modeling an adsorption/absorption process, the “±” in Eq. [1] becomes positive (+).

The first derivative of the Peleg equation, Eq. [2], gives the momentary sorption rate (*S*).

$$ S = \frac{dM(t)}{dt} = \frac{K_1}{(K_1 + K_2 t)^2} $$

The Peleg rate constant, *K₁*, inversely relates to the initial sorption rate (i.e., *S* at time zero, or *t* = *t₀*).

$$ S_0 = \frac{1}{K_1} $$

The Peleg capacity constant, *K₂*, relates to the equilibrium moisture content, *Mₑ*, as time approaches infinity.

$$ Mₑ = M_0 + \frac{1}{K_2} $$

The model was fit to our data using Eq. [1] and non-linear model fitting procedures in JMP (SAS Institute, Cary, NC) to generate values for *K₁* and *K₂*. *M₀* was calculated directly from our data. To verify the accuracy of the Peleg model for predicting water uptake in *R. asiaticus*, Eq. [1] was rearranged to its linear form, Eq. [5], with linear regression used to provide a coefficient of variance (*R*²).

$$ \frac{t}{M(t) - M₀} = K_1 + K_2 t $$

The hydration ratio, *R*, at a given value of *M(t)* may be calculated by Eq. [6].

$$ R = \frac{M(t) - M₀}{Mₑ - M₀} $$

Once a value for a desired hydration ratio is determined, *R₁*, it may be subsequently used to estimate the time necessary to achieve other hydration ratios, *R₂*. 

Received for publication 6 Sept. 2011. Accepted for publication 19 Nov. 2011.

This work constitutes a portion of a dissertation submitted by C.B. Cerveny to the Graduate Faculty of Cornell University in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

We thank the Fred C. Gloeckner and Post-Schenkel Foundations for grant support, The California Flower Bulb Co. and The Flower Fields at Carlsbad Ranch for donation of plant materials, and the Cornell Statistical Consulting Unit for assistance with data analysis. We also thank Joanna Blaszczak, Suzana Markolovic, and Siddharth Ramshankar for their assistance with data collection.

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at 1375 mg L\(^{-1}\) metallic copper. A series of incubators were used to achieve constant temperature during the hydration period. Roots were planted four per pot on 7 Oct. in 15-cm diameter azalea pots using a commercial potting mix (Sun Gro LC1; Sun Gro Horticulture Canada Ltd., Vancouver, British Columbia, Canada) with crowns covered by \(\approx 2\) cm of substrate. Planted roots were moistened with tap water and then held at 5 \(\degree\)C for 4 weeks to allow root establishment before growing in a 15 \(\degree\)C set point temperature greenhouse starting on 4 Nov. The plants were organized in a completely randomized design with six replicate pots of four sub-samples (pooled) per treatment.

Plants were evaluated after 4 weeks in the greenhouse for percent survival (any visible growth), plant height (substrate line to tallest leaf), and shoot dry weight (severed at soil level and dried 3 d at 70 \(\degree\)C).

**Greenhouse Expt. 2.** Dried ‘Tecolote Pink’ roots were handled in a similar manner as Expt. 1, except the 24-h hydration temperatures were 5, 10, 17, 20, 25, 30, or 35 \(\degree\)C. Treatments were initiated on 8 Dec., and roots were planted on 10 Dec., held for 5 weeks (as described previously), and then moved to the greenhouse on 14 Jan. 2009. Plants were arranged in a completely randomized design with nine replicate pots of four sub-samples (pooled) per treatment. Percent survival and plant height were evaluated after 4 weeks in the greenhouse. After 11 weeks, plants were evaluated for number of flowering stems (stems with at least one flower) and shoot dry weight (as in Expt. 1).

**Greenhouse Expt. 3.** The cultivars and tissue sources were increased in Expt. 3. *R. asiaticus* ‘Tecolote Pink’ and ‘Labelle Cream’ were obtained from 2007–2008 growing seasons in southern California (same source as described previously) and France (unknown origin), respectively. Additionally ‘Tecolote Red’ and ‘Tecolote White’ from the 2008–2009 growing season were also obtained from southern California. Roots were held at 15 \(\degree\)C and \(\approx 50\%\) relative humidity until treatments were initiated on 28 Sept. 2009. Roots were hydrated at 5, 15, 25, or 35 \(\degree\)C for 24 h, treated with a copper biocide, and planted as described previously. After 6 weeks cooling at 5 \(\degree\)C, plants were moved to the greenhouse on 9 Nov. Percent survival, plant size (mean of height and two cross canopy diameter measurements), and shoot dry weight (as described previously) were calculated after 4 weeks in the greenhouse (7 Dec. 2009). A visual plant quality ranking was also assigned on a 1–5 scale: 1 = poor quality, one to two leaves, diseased and/or dying; 2 = unacceptable quality, slightly more growth, three to six leaves; 3 = acceptable quality, seven to 10 leaves, non-uniform growth; 4 = moderate quality, uniform growth, greater than 10 leaves; 5 = best quality, ideal size and shape, greater than 10 leaves.

For greenhouse Expts. 1–3, all measurements and subsequent analyses were conducted on plants showing visible growth. All data were analyzed using standard least squares in JMP (SAS Institute, Cary, NC) with specific models as indicated for each experiment.

**Results and Discussion**

Assessment of the model. *Ranunculus asiaticus* exhibited typical absorption behavior at all temperatures tested, initially absorbing water rapidly and slowing as moisture content approached equilibrium (Fig. 1). The hydration rate was faster at warmer temperatures but to a lower equilibrium than when hydrated cooler. To assess the model fit, the linear form of the Peleg model [Eq. (5)] is shown in Figure 2 with coefficient of variance (\(R^2\)) values from 0.96 to 0.99. It should be noted that Eq. [5] was only used to obtain \(R^2\) terms (Fig. 2); thus, values for \(K_1\) and \(K_2\) used for further analyses were generated by our statistical software package using the Peleg model [Eq. (1)] in standard format. When comparing our calculated to observed moisture content in hydrating *R. asiaticus* roots, the model’s predicted values were within 3% of our observed values at both 12 and 24 h hydration (Table 1). The Peleg rate constant, \(K_1\), is related to the mass flux (rate of weight change) in that a lower value of \(K_1\) indicates a faster initial water absorption rate. In our experiment, as temperature increased, \(K_1\) decreased, corresponding to faster initial water absorption at higher temperatures (Table 1). The influence of temperature on the Peleg rate constant is shown in Figure 3 through the linearized Arrhenius equation with an \(R^2\) of 0.91:
where $k_0$ is a constant, the pre-exponential factor, or prefactor (h%); $E_a$ is the activation energy (kJ mol$^{-1}$); $R_g$ is the universal gas constant (8.314 kJ mol$^{-1}$K); and $T$ is the absolute temperature (K). To determine the activation energy, the slope of the line generated in Figure 3 is multiplied by $-R_g$. The resultant activation energy for *R. asiaticus* hydration is 24.8 kJ mol$^{-1}$, comparable to other organic substances modeled using the Peleg equation; the resultant activation energies were 29.4 kJ mol$^{-1}$ for apricot kernels, 14.8 or 59.3 kJ mol$^{-1}$ for chickpeas above or below 55 °C, respectively, and 39.2 or 60.4 kJ mol$^{-1}$ for roasted or raw lupin seeds, respectively (Celen et al., 2008; Solomon, 2007, 2009; Turhan et al., 2002).

The Peleg capacity constant, $K_2$, is inversely related to maximum water absorption; therefore, the lower the $K_2$, the higher the absorption capacity. As hydration temperature increased, $K_2$ for *R. asiaticus* also increased, which indicates lower equilibrium moisture content at 30 °C than at 5 °C (Fig. 4; Table 1). Similar trends in $K_2$ values were observed when chickpea and kidney bean (*Phaseolus vulgaris* L.) were soaked at increasing temperatures (Abu-Ghannam and McKenna, 1997; Turhan et al., 2002).

Water uptake was faster in warmer water. Because growth or decay will alter the system before 100% saturation is reached, we used 75% of $M_0$ as the threshold for hydration time measurements. The time to achieve 75% of $M_0$ was 30 h at 5 °C and decreased to 15.0 h at 20 °C and 6 h at 35 °C (Table 2). Hydrating roots for 12 h at 5, 20, or 35 °C resulted in moisture contents within 57%, 71%, or 82% of $M_0$, respectively (Tables 1 and 2). Increasing the soaking time to 24 h increased moisture content another 16%, 13%, or 7% to 73%, 84%, and 89% of $M_0$ when hydrated at 5, 20, or 35 °C, respectively (Table 1). At room temperature, soaking for 24 h before potting was considered sufficient to hydrate the tubers. We did not investigate the influence of soaking duration on subsequent plant performance.

**Fig. 3.** Arrhenius plot for the Peleg rate constant, $K_1$, during hydration of *R. asiaticus* roots. Symbols represent means ± se.

**Fig. 4.** Influence of temperature on the Peleg capacity constant, $K_2$, during hydration of *R. asiaticus* roots. Symbols represent means ± se.

| Temperature (°C) | Calculated sorption rate (%/h) | $t_f$ (h) |
|------------------|-------------------------------|---------|
|                  | Initial After 12 h After 24 h | $R_{0.5}$ $R_{0.75}$ $R_{0.85}$ $R_{0.95}$ |
| 5                | 22.3 5.1 2.2                 | 9.4 30.0 57.5 195.0 |
| 20               | 41.0 4.1 1.5                 | 9.6 15.0 28.7 97.4 |
| 35               | 63.0 3.0 0.9                 | 2.6 9.6 16.5 56.1 |

*See Eq. [3].

$^a$See Eq. [6], Eq. [7]. $t_f$ is the time (h) to reach a given hydration level.
It is therefore possible that the same phenomenon, rapid imbibition, is responsible for decreased survival in *R. asiaticus* at both high and low temperatures. Further investigations are necessary to determine if mixing an osmotic or matric inhibitor such as polyethylene glycol (PEG) into the *R. asiaticus* hydration solution will alleviate symptoms observed at potentially damaging temperatures. Because the PEG solution would reduce the rate of water uptake, it may serve to further determine a mechanism for damage outside of the optimum range for growth. It may also be useful to measure solutes (i.e., sugars or ions) in the hydration solution during imbibition, which could further support membrane damage as the reason for reduced viability.

Priming [exposure to periods of brief hydration and re-drying at temperatures above which CI occurs (25 °C)] of soybean seeds (*Glycine max* L.) reduced the cellular damage when seeds were later soaked in 4 °C water (Tilden and West, 1985). In one preliminary experiment, dry *R. asiaticus* roots had improved sprouting when hydrated 24 h at 25 °C, allowed to re-dry 1 week at 25 °C, and then rehydrated 24 h at 25 °C compared with those given a single hydration period (24 h at 25 °C) (Cerveny and Miller, unpublished data). It is not known if priming *R. asiaticus* roots at moderate temperature (20 °C) would alleviate the observed problems when hydrating at low temperatures.

It is interesting that the trend for plant quality parameters was generally consistent with trends in plant survival for the first 4 weeks of growth (Tables 3 to 5). This effect appears transient, however, because those observed parameters were not significantly different at 11 weeks' growth. The number of flowers was unaffected by hydration temperature, presumably because initiation occurs after sprouting in the *R. asiaticus* growth cycle (Kamenetsky et al., 2005). Foliar dry weight followed a similar quadratic trend to plant size in the greenhouse.

As a result of noticeable growth habit variations among individual *R. asiaticus* plants, we were unsure if our measured parameters were adequate to describe plant growth in Expt. 1; thus, additional parameters were added with subsequent experiments. However, because plant height, size, and visual quality were not significantly different at 11 weeks' growth, the observed problems when hydrating at low temperatures are likely to be important in future studies to determine if low hydration temperature results in a developmental delay after sprouting rather than simply a change in plant size or flower number at maturity. This information could be particularly important to forcers of geophytes.
consistently supported by shoot dry weight values, it seems the measured parameters were appropriate quantifiers for \textit{R. asiaticus} growth (Fig. 5; Tables 3 to 5).

Water temperature monitoring is an important factor when hydrating \textit{R. asiaticus} and should be maintained between 15 and 25 °C. The common cultural recommendation to “hydrate roots via slowly running water” (De Hertogh, 1996; Umiel and Hagiladi, 1999) may result in poor results if the tap water is too cold. For instance, the temperature of tap water in our laboratory was seasonally as low as 5 °C and its maximum was 15 °C; therefore, the entire range of our tap water temperature may be too cold for optimum hydration of \textit{R. asiaticus}. It is also possible for tap water to be too warm if the plumbing is exposed to sunlight or other heat sources, like in a greenhouse setting.

In commercial production, missing plants are expensive to replace; therefore, plant survival is probably the most important variable for determining treatment success. Once survival is optimized, quality parameters become increasingly important. Our results demonstrate that a hydration temperature between 15 and 25 °C is necessary to obtain the best quality when growing \textit{Ranunculus asiaticus} from its dried tuberous roots.

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