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Storage Temperature and Moisture Content Affect Respiration and Survival of *Ranunculus asiaticus* Dry Tuberous Roots

Christopher B. Cerveny and William B. Miller[1]
Department of Horticulture, Cornell University, 134 Plant Science, Ithaca, NY 14853

Alan G. Taylor
Department of Horticulture, Cornell University, Geneva, NY 14456

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***Abstract. Ranunculus asiaticus* (L.) is an ornamental geophyte with some commercial production challenges presumed to be related to the storage of its desiccation tolerant tuberous roots (TRs). We investigated the influence of temperature and relative humidity during storage on viability of *R. asiaticus* TRs. The TRs were stored in specialized chambers for controlling relative humidity under flow-through or closed systems. In the flow-through system, air was bubbled through glycerol–water solutions to create relative humidities of 20%, 40%, 60%, 80%, or 100% and then passed through storage chambers held at 5, 20, or 35 °C for up to 20 weeks. In closed storage, tissue was equilibrated to a given moisture content (fresh basis) at 15 °C by suspending TRs over glycerol–water solutions (35%, 60%, or 85% relative humidity) with fans to circulate air. These containers were closed for 4 weeks and then tissue was transferred to sealed jars for up to 17 weeks at 5 or 25 °C. In both systems, TRs held with elevated temperature and relative humidity had the largest decrease in percent survival when planted after storage. Flow-through storage gave greater variability in TRs moisture content than closed storage. Tuberous roots at 25 °C had higher respiration rates than at 5 °C under closed storage; elevated moisture content also led to increased respiration. From these results it can be concluded that *R. asiaticus* dry TRs should be stored cool and dry for long-term viability.**

Ranunculus asiaticus (L.) is an ornamental geophyte prized for its showy flowers and desiccation tolerant, dry TRs. *R. asiaticus* is native to the eastern Mediterranean in areas with a cool, wet winter and hot, dry summer. In their native and similar climates, the plants are commonly grown in the field for one or more seasons before the TRs are harvested, dried, and sold to flower growers worldwide. Although *R. asiaticus* grown from transplanted dry TRs reportedly produce a faster and more profuse flowering crop than when grown from seed (Meynet, 1993), inconsistent sprouting, poor uniformity, and abnormal growth habit are common problems after storage (M.A. Mellano, personal communication).

The influence of temperature and relative humidity on the aging of seeds has been well documented (Justice and Bass, 1978). The general recommendation is that the drier and cooler the storage environment, the longer seeds retain the ability to germinate (Priestley, 1986). Analogies are made throughout this article on seed storage as a well-studied system for low temperature and low moisture content storage. In this article, "seed storage" is for those species with orthodox storage behavior, those that can withstand desiccation (Copeland and McDonald, 2001).

Artificial aging of seeds has been recognized as a useful predictor of storability; those with reduced vigor under accelerated aging treatments usually respond similarly under long-term open storage (Priestley, 1986). In accelerated aging experiments, seeds are stored much warmer than is typical, at 35 to 45 °C, and up to 100% relative humidity, which lowers viability in seeds in a matter of days or weeks as compared with years in naturally aging tissues (Bewley and Black, 1994). Traditionally, *R. asiaticus* dry TRs are handled by distributors who market other ornamental geophytes and typically have limited storage options at their disposal. These conditions are commonly cool and moist, appropriate for (*Lilium* spp. L.) bulbs; cool and dry such as with gladiolus (*Gladiolus* spp. L.); or room temperature and dry along with other dry-packed bulbs such as calla lilies (*Zantedeschia* sp. Koch.). Meynet (1993) suggested that *R. asiaticus* TRs should be stored at 15 to 25 °C and 50% relative humidity, which is common in the industry (M.A. Mellano and Y. Liberman, personal communication), but this range has not been scientifically tested. It is unclear which of the previously mentioned storage conditions are most appropriate for long-term *R. asiaticus* storage. The purpose of this research was to develop a storage protocol for *R. asiaticus* dried TRs by testing a range of storage moistures and temperatures and observing the influence on respiration and subsequent growth.

Materials and Methods

Expt. 1: Dynamic storage. In this experiment, a flow-through system was used to control relative humidity and temperature during storage. Dried *Ranunculus asiaticus* 'Tecolote Merlot' (M07) TRs were obtained in Apr. 2008 from a commercial grower (California Flowerbulb Co., Carlsbad, CA). The TRs originated from commercial plantings in the 2006–2007 winter growing season. On 10 Apr., TRs were placed into modified storage at 5, 20, or 35 °C for 5, 10, or 20 weeks. This was a factorial design with three temperatures, three storage periods, and five relative humidity treatments. Storage chambers consisted of 5-gallon plastic pails into which humidified air was introduced. This was generated by pumping air at 500 mL·min^{-1} through glycerol–water solutions mixed to desired ratios as outlined by Forney and Brandl (1992). Two 2-L treatment jars, each containing 1500 mL of glycerol–water solution, were plumbed in series to provide the desired relative humidity to the storage pails (Fig. 1). The specific gravity in the treatment jars averaged 1.221, 1.218, 1.178, 1.094, or 1.000 over the course of the experiment, which provided relative humidity of ≈20%, 40%, 60%, 80%, or 100%, respectively. There was a single replicate pail per humidity treatment. Relative humidity in each pail was verified using HOBO Pro v2 data loggers (Model U23-002; Onset Computer Corp., Bourne, MA) and was determined to be within ± 5% across all treatments. After 5, 10, or 20 weeks, storage pails were opened and 20 TRs from each humidity treatment were removed for moisture content determination (dried in a drying oven at 70 °C until constant weight was achieved, calculated on a fresh weight basis) and the pails resealed. No TRs were planted after 5 weeks, but, after 10 weeks' storage, 48 TRs from each temperature and humidity treatment were removed for planting. These TRs were placed in mesh bags suspended ≈2.5 cm over saturated potting media for 3 d at 5 °C to prepare samples for hydration. This treatment was done to allow the very low moisture content TRs to receive some equilibration to higher moisture before submerging.

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[1]To whom reprint requests should be addressed; e-mail wbm8@cornell.edu.

It is not known if this treatment is necessary in *R. asiaticus* but was included as a safeguard to prevent possible hydration injury from biasing treatment effects (Copeland and McDonald, 2001). The TRs were then submerged in tap water for 24 h at room temperature and provided a 20-min soak in a commercial fungicide (Captan, N-trichloromethylthio cyclohexene-1,2-dicarboximide; Southern Agricultural Insecticides Inc., Hendersonville, NC) at 2.6 g a.i./L. On 24 June, TRs were planted in 7.5-cm square pots using a commercial potting mix (Sun Gro LC1; Sun Gro Horticulture Canada Ltd., Vancouver, British Columbia, Canada) with crowns covered ≈2 cm. Pots were watered initially and as needed throughout a 5-week cooling period provided at 5 °C, which allowed some root establishment before moving to the greenhouse on 1 Aug. (De Hertogh, 1996). After 2.5 weeks of growth (on 18 Aug.), plants were evaluated for percent survival (any visible growth).

Tuberous roots stored for 20 weeks were handled in the same manner as those stored 10 weeks, except they were planted on 2 Sept., given 4 weeks storage at 5 °C, and were moved to the greenhouse with a 20 °C constant set point temperature on 3 Oct. Percent survival was calculated after 4 weeks on 30 Oct. In both 10- and 20-week plantings, pots were arranged in a completely randomized design with two 24 subsample replicates (averaged) per treatment. Data were analyzed using standard least squares in JMP (SAS Institute, Cary, NC).

Expt. 2: Closed storage. Dried TRs of *Ranunculus asiaticus* 'Labelle Cream' (L08) were obtained in Dec. 2008 from a commercial producer in France (unknown origin) grown during the 2007–2008 season. 'Tecolote Pink' (P09) and 'Tecolote White' (W09) were obtained in Aug. 2009 from the 2008–2009 growing season in southern California (California Flowerbulb Co., Carlsbad, CA). Tuberous roots were stored at 15 °C and ≈50% relative humidity until treatments were initiated.

In this experiment, TRs were held in a closed system as opposed to the flow-through system described in Expt. 1. Because moisture content of stored TRs increased over the entire 20 weeks of Expt. 1 (Fig. 3), this experiment involved equilibrating TRs to different moisture contents for 4 weeks before randomly assigning TRs to temperature treatments. This created uniformity in TR moisture content at the various temperatures tested and allowed us to observe a more controlled interaction between temperature and TR moisture level (Priestley, 1986).

Humidity equilibration chambers were constructed using two 5-gallon plastic pails stacked inside one another (Fig. 2). Pail A contained 2 L of glycerol–water solution mixed to the desired specific gravity (see subsequently). Pail B had the bottom removed and replaced with a plastic grid along with a 12-V DC fan (Model 273-240; Radio Shack Corp. Ft. Worth, TX) on each end. Pail B was inserted into Pail A and a rubber gasket around the perimeter (X-TREME™ rubber weather seal; Thermwell Products Co., Mahwah, NJ) created an airtight seal between the pails. *R. asiaticus* TRs and two data loggers were then placed into Pail B and the top sealed using a tightly fitting lid and duct tape. The fans were powered using a power supply (Model 22-508; Radio Shack Corp.) connected to wires extending out of a gasket-sealed 2-mm port in the pail lid. In this experiment, TRs were stored for 4 weeks (at 15 °C) suspended over a glycerol–water solution with headspace equilibrium relative humidity of 35%, 60%, or 85%. There was a single replicate equilibration chamber for each humidity treatment. The humidity treatments were verified with HOBO Pro v2 data loggers (as previously mentioned) and were within ± 5% across all treatments. The specific gravity of the glycerol–water solutions (the same in both rounds of the experiment) was 1.240, 1.190, or 1.126 for the 35%, 60%, or 85% relative humidity chambers, respectively. After 4 weeks equilibration, TRs were removed from the humidity chambers and were randomly assigned to one of two temperature treatments, 5 or 25 °C, for 16 weeks storage. Four subsample TRs were sealed in 0.5-L jars with six replicate jars per treatment (temperature and moisture content) per cultivar. This was a factorial design with three humidity treatments and two temperatures. Additionally, 10 TRs from each humidity chamber per cultivar were sacrificed for moisture content calculation on a fresh weight basis [(water weight/fresh weight) × 100]. The moisture content of TRs stored at 35%, 60%, or 85% relative humidity was 6.9%, 10.1%, or 18.4%, respectively, and was not different between rounds of the experiment. The total number of TRs under storage was 154 per cultivar.

On removal from 16-week storage treatments (not including 4 weeks equilibration), TRs were placed in mesh bags and held over saturated potting media for 3 d at 5 °C to prepare samples for hydration as in Expt. 1. Tuberous roots were then submerged in tap water for 24 h at 25 °C and provided a 5-min soak in a copper sulfate biocide (Phyton-27; Phyton Corp., New Hope, MN) at 1375 $mg \cdot L^{-1}$ metallic copper. They were planted, four per pot, in 15-cm diameter azalea pots using a commercial potting mix (as described previously) with crowns covered ≈2 cm on 6 or 21 Jan. for Rounds 1 and 2, respectively. Planted TRs were moistened with tap water and then given a 4-week cooling period at 5 °C to allow some root establishment before growing in a 15 °C set point temperature greenhouse starting on 4 or 16 Feb. for Rounds 1 or 2, respectively. There were six replicate pots (one per block) of four subsamples (pooled) per treatment.

After 4 weeks in the greenhouse, data on percent survival (any visible growth), plant size (mean of height and two cross-canopy diameter measurements), and foliar dry weight (severed at soil line and dried 3 d at

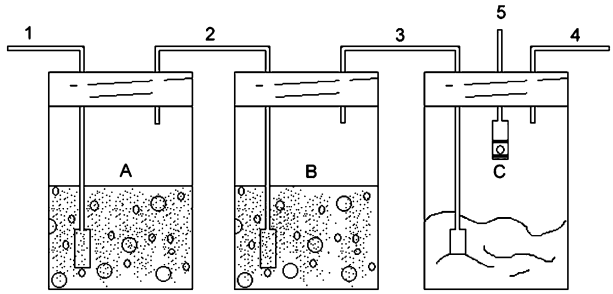

Fig. 1. Flow-through humidity chambers used in Expt. 1. Air was pumped through Line 1 into one of two jars (**A–B**) containing glycerol and water mixed to the desired specific gravity. Humidified air then passed through Line 2, then Line 3, and finally Line 4, leading into the storage vessels. Jar C contained a paper towel to absorb any condensation/liquid and a relative humidity/temperature monitoring probe (5). (Adapted from Forney and Brandl, 1992.)

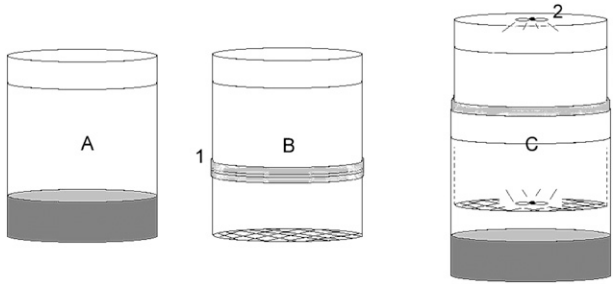

Fig. 2. Closed humidity chambers used in Expt. 2. Pail A contained glycerol and water solutions mixed to the desired specific gravity. Pail B had a tightly fitting lid, a microfan at each end (2), and the bottom replaced with a plastic screen. Pail B was inserted into Pail A to create the storage chamber (C). A rubberized gasket (1) created an airtight fit between pails; the lid of Pail B was also reinforced with duct tape (not pictured).

70 °C) were collected. A visual plant quality ranking was also assessed using a 1 to 5 scale: 1 = poor quality, one to two leaves, diseased and/or dying; 2 = poor 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; and 5 = best quality, ideal size and shape, greater than 10 leaves.

For both rounds, any experimental unit that returned a zero value for survival was excluded from further calculations; therefore, all remaining measurements and subsequent analyses were conducted only on plants showing visible growth. All data were analyzed using standard least squares in JMP (SAS Institute).

Expt. 3: Respiration under closed storage. In the third experiment, CO_2 accumulation was measured in the headspace of TRs under similar conditions as in Expt. 2. Closed humidity chambers were set up as previously described with TRs equilibrated 4 weeks at 15 °C starting on 22 Oct. An additional cultivar, Tecolote Pink (P08) (from the 2007–2008 growing season) was included along with the cultivars used in Expt. 2 (L08, P09, and W09). After 4 weeks (17 Nov.), pails were opened and TRs randomly assigned to 5 or 25 °C storage. Tuberous roots were placed in 100-mL jars with airtight lids fitted with rubber septa. There were two subsample TRs per jar and six replicate jars per treatment per cultivar. In this experiment, moisture content was determined with five TRs per humidity chamber per cultivar and then at each sampling date (see subsequently). Dry weights were collected after freeze-drying samples. The moisture content {calculated on a fresh weight basis: [(water weight/fresh weight) × 100]} of TRs in this round (not significantly different between cultivars or sampling dates) was 1.9%, 4.9%, or 14.1% for the 35%, 60%, or 85% relative humidity treatments, respectively.

Gas samples were collected and measured for CO_2 (see subsequently) after 4, 12, and 17 weeks of storage in jars. At each sampling date, two jars from each treatment and cultivar were opened, flushed with air, and resealed. These jars were sampled again 3 d later for CO_2 and then TRs were analyzed for moisture content (see previous paragraph). Carbon dioxide measurements were made on 1-mL headspace samples injected into a nitrogen stream passing through a CA-10a carbon dioxide analyzer (Sable Systems Intl., Las Vegas, NV). A calibration curve was created using increasing volumes of a CO_2 standard to establish a conversion factor for peak areas generated by the CA-10 software. Atmospheric CO_2 concentration was obtained by including four empty jars per sampling period with their mean value subtracted from total CO_2 evolved. To calculate respiration rate, the fresh weight of tissue in each jar was subtracted from the headspace volume (assuming a tissue density of 1 g·cm^3) and data expressed as milliliter CO_2 evolved per kilogram of TR fresh weight per day. Data from this factorial experiment (three relative humidities and two temperatures) were analyzed using standard least squares in JMP (SAS Institute).

Results

Expt. 1: Dynamic storage. The moisture content of TRs under modified storage had a significant temperature by relative humidity by time interaction (Table 1). Tuberous root moisture content tended to increase with relative humidity as storage duration and temperature increased (Fig. 3). For example, moisture content of TRs was similar among humidity treatments after 5 weeks storage at 5 °C but increased with relative humidity after 20 weeks, whereas those held at 20 °C had increased moisture content with humidity at all sampling dates.

The temperature by relative humidity by time interaction was significant for percent survival (Table 1). After 10 weeks storage, a significant reduction in percent survival was observed after storage at 35 °C, especially when relative humidity was 80% or higher (Fig. 4). Percent survival was generally similar across humidity treatments at cooler storage temperatures but was slightly reduced when stored at 20 °C and 100% relative humidity (Fig. 4). After 20 weeks storage, percent survival decreased as relative humidity increased at both 20 and 35 °C, and those stored warmer showed greater survival loss at each humidity level. Tuberous roots stored 20 weeks at 5 °C had similar survival across humidity treatments.

Expt. 2: Closed storage. Results from both rounds of the experiment were similar; therefore, only results from Round 2 are presented. There were no significant differences among cultivars for percent survival or visual quality (Table 2). The moisture content by temperature during storage interaction was significant for percent survival (Table 2). Tuberous roots with 22.6% moisture content had zero plants survive when stored at 25 °C, whereas all other treatments had similar survival (92.3%). No differences in visual quality, plant size, or foliar dry weight (averaged 4, 11.3 cm, or 0.66 g, respectively) were shown with regard to storage moisture content or temperature (Table 2). Plant size and foliar dry weight were different among cultivars (Table 2). With regard to plant size, L08 was significantly smaller than W09 (11.3 vs. 10.7 cm, respectively); however, both were similar to P09, which averaged 11.0 cm per plant. Foliar dry weight followed a similar trend except all three cultivars were significantly different at 0.82, 0.67, and 0.50 g for W09, P09, and, L08, respectively.

Expt. 3: Respiration during closed storage. Respiration rates were similar for TRs with 1.9% or 4.9% moisture regardless of storage temperature (Fig. 5). Increasing TR moisture content to 14.1% increased respiration rate, which was at least 12.5 times higher with TRs held at 25 °C than at 5 °C.

Table 1. Expt. 1: Significance, indicated by *P* value, of temperature, relative humidity during storage, time (weeks of storage), and their interaction on *Ranunculus asiaticus* tuberous root moisture content (MC).[z]

Source	MC	Percent survival
Temperature (T)	<0.001	<0.001
RH	<0.001	<0.001
T × RH	<0.001	<0.001
Time (W)	<0.001	0.001
T × W	<0.001	NS
RH × W	<0.001	0.013
T × RH × W	<0.001	0.042

[z]Percent survival was any visible growth after 3 weeks in the greenhouse after up to 20 weeks modified storage.
NS Nonsignificant at $\alpha = 0.05$.
RH = relative humidity.

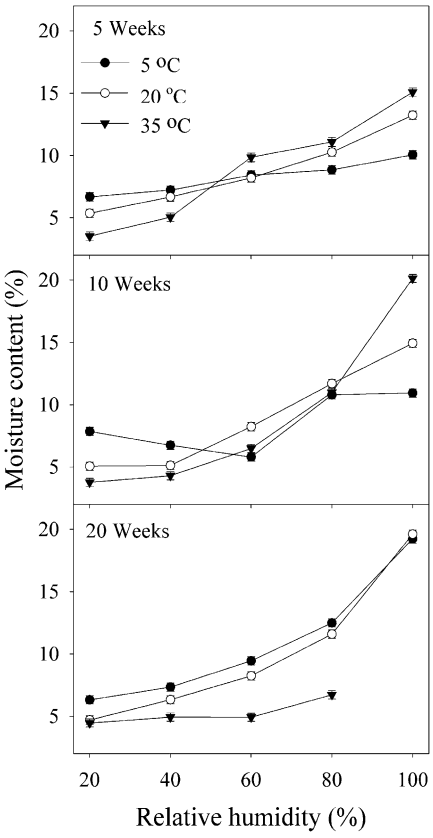

Fig. 3. Expt. 1: Dynamic storage. Fresh basis moisture content of *R. asiaticus* 'Tecolote Merlot' tuberous roots stored 5, 10, or 20 weeks under increasing temperature and relative humidity conditions. Data are means ± SE (bars not visible are within range of data symbol).

Respiration rates were not different among cultivars or sampling dates (Table 3).

Discussion

To establish proper storage protocols for *R. asiaticus* dried TRs, modified temperature and relative humidity treatments were imposed under dynamic (flow-through) or closed storage systems. In both systems, TR moisture content (or humidity) interacted

with time, which indicated elevated moisture and temperature were detrimental to long-term viability. When comparing these results with data from the third experiment, TRs stored with the highest moisture content also had the highest respiration rate (Fig. 5).

In an effort to establish a paradigm for long-term open storage of seed, seeds are often exposed to accelerated aging treatments (35 to 45 °C and up to 100% relative humidity), which provide viability loss in a much shorter duration than under natural aging (Copeland and McDonald, 2001). Priestley (1986) cautioned against using accelerated aging studies to replace the need for long-term verification of results but suggested these treatments give some approximation to what happens with natural aging. When pea seeds (*Pisum sativum* L.) were given accelerated aging treatments (37 °C and 100% relative humidity), respiration rate increased and vigor decreased compared with those stored at 20 °C (Ozga et al., 2004). Storage of *R. asiaticus* TRs at 5 to 35 °C and up to 100% relative humidity was effectively "accelerated aging" of the TRs. When stored at 35 °C, viability was completely lost within 10 weeks at 100% relative humidity or 20 weeks at 80% relative humidity. Tuberous roots stored at 5 °C showed mildly elevated respiration rates with higher moisture content, yet did not exhibit significant survival loss in the greenhouse (Expts. 2 and 3). We speculate that TRs stored at 5 °C under elevated moisture would eventually have shown reduced viability if the experiment duration were longer, especially when considering the change in moisture content with time, temperature, and relative humidity during storage (Fig. 3).

Long-term viability of seeds is greatly influenced by temperature and moisture content during storage; for example, it has been noted that the storage life doubles for each 1% decrease in seed moisture content and/or 5.6 °C decrease in storage temperature (Bewley and Black, 1994). The range for acceptable humidity and temperature depends on the tolerance of the species to both extreme drying and low temperature. The relationship between relative humidity and seed moisture content is usually expressed by moisture sorption isotherms, which are obtained by measuring equilibrium moisture content as a function of relative humidity at constant temperature (Priestley, 1986). These curves have a characteristic sigmoid shape that permits expression of three distinct regions of hydration, or "zones." In Zone I, tissue moisture content increases rapidly with rising humidity and then slows in Zone II. In Zone III, moisture content again rises rapidly with increased humidity. Internal composition of seeds (starch or oil) can affect the equilibrium moisture content, but the inflection points between zones are relatively similar among seeds (Priestley, 1986).

In our experiments, a similar trend in the upper half of a sigmoid-shaped curve emerged when TRs were stored under increasing relative humidity (Fig. 3); however, the lowest relative humidity imposed was 20%, which is in the upper region of Zone I water binding in seed moisture isotherms (Priestley, 1986). Moisture content of TRs under dynamic storage generally rose with increasing relative humidity and temperature; however, after 20 weeks at 35 °C, moisture content was similar among humidity treatments. Because respiration was shown to be greater as temperature and humidity increased in the closed system (Fig. 5), this probably resulted in a loss of dry weight as the carbon substrate in the TRs was consumed (Hopkins, 1999), which appeared as a relative decline in moisture content at higher humidity over time (10 vs. 20 weeks). Although relative humidity is the chief contributing factor to decreased longevity in stored seeds, this effect is largely explained through its influence on seed moisture content (Priestley, 1986). Priestley (1986) diagramed evidence of cellular activity at various levels of hydration in seeds. He concluded that seed respiration is only feasible in upper Zone II or in Zone III but indicated these data are often confounded by various microorganisms living on the seeds, which become more active as moisture level increases and can contribute significantly to the respiratory gas exchange in the storage environment. *R. asiaticus* is known for having infections of various root-rot pathogens during storage, thus inclusion of biocide treatments before planting (Meynet, 1993). In our studies, we were not able to distinguish the source of respiration. It is possible that CO_2 produced during storage of *R. asiaticus* TRs was the result of respiring microorganisms colonizing the TRs rather than from the tissue itself. If further investigations of *Ranunculus* respiration are warranted, it may be necessary

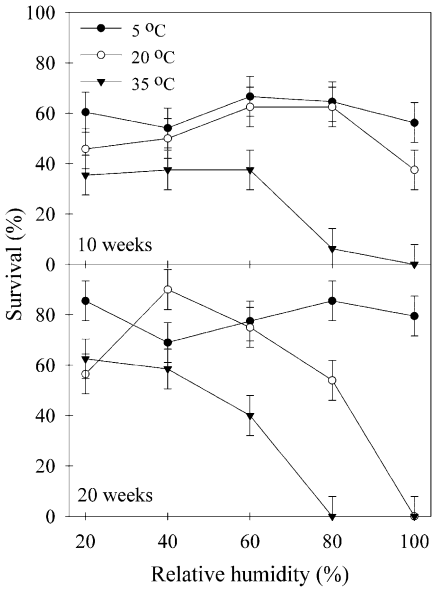

Fig. 4. Expt. 1: Dynamic storage. Influence of time, temperature, and storage humidity on percent survival of *Ranunculus asiaticus* 'Tecolote Merlot' tuberous roots. Data are means ± SE.

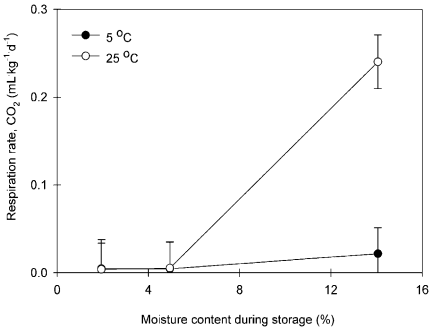

Fig. 5. Expt. 3: Influence of tissue moisture content (fresh basis) on respiration rate (CO_2 evolution) of *R. asiaticus* tuberous roots held at 5 or 25 °C. Data are means averaged over 17 weeks storage ± SE.

Table 2. Expt. 2: Significance, indicated by *P* value, of cultivar, moisture content, temperature, and their interaction during storage on the percent survival, visual quality ranking, plant size, and foliar dry weight of *R. asiaticus* tuberous roots after 20 weeks specialized storage and 4 weeks growth in the greenhouse.

Source	Percent survival	Visual quality ranking	Plant size	Foliar dry wt
Cultivar (C)	NS	NS	0.001	<0.001
Moisture content (MC)	<0.001	NS	NS	NS
C × MC	NS	NS	NS	NS
Temperature (T)	<0.001	NS	NS	NS
C × T	NS	NS	NS	NS
MC × T	<0.001	NS	NS	NS
C × MC × T	NS	NS	NS	NS

NS Nonsignificant at $\alpha = 0.05$.

Table 3. Expt. 3: Significance, indicated by *P* value, of time (weeks of storage), cultivar, temperature, moisture content, and their interaction on respiration rate (CO_2 evolution) of *R. asiaticus* tuberous roots during specialized storage.

Source	Respiration rate
Weeks (W)	NS
Cultivar (C)	NS
W × C	NS
Temperature (T)	0.005
W × T	NS
C × T	NS
W × C × T	NS
Moisture Content (MC)	<0.001
W × MC	NS
C × MC	NS
W × C × MC	NS
T × MC	<0.001
W × T × MC	NS
C × T × MC	NS
W × C × T × MC	NS

NS Nonsignificant at $\alpha = 0.05$.

to disinfect TRs before storage. This may isolate the source of respiration gas to the tissue.

In a recent study, when *R. asiaticus* TRs were stored for 1 year at 2 °C under either open or modified atmosphere storage (2% O_2 and 4% CO_2), percent survival was maintained at 90%, which was significantly greater than those under open storage at room temperature (ranged ≈15 to 23 °C) (Beruto et al., 2009). It was proposed that the nonsignificant influence of atmosphere modification on viability was the result of low metabolism during storage. This hypothesis is further upheld by the low respiration rates of our tissue stored at 5 °C. The long-term results under natural aging presented by Beruto et al. (2009) support our findings over the relative short-term.

Under dynamic storage, the moisture content of TRs did not appear to reach equilibrium moisture levels across temperature treatments over the 20 weeks of storage, but rather increased with increasing temperature (Fig. 3). Thus, when redesigning the experiment for closed storage, TRs were first equilibrated to a moisture content that was predicted to cause damage or not. This treatment allowed much more uniform response to relative humidity at the temperatures tested and did not change from the start of temperature treatments to completion. Under commercial conditions, it is difficult to speculate exactly how episodes of high or low humidity would impact the overall storability of *R. asiaticus*, but the general tenet emerges that dry storage is better than humid.

It should be noted that in Expt. 1, average plant survival across all treatments was lower after 10 weeks than 20 weeks storage. We speculate this was attributable, in part, to the need to grow *R. asiaticus* plants in late summer when greenhouse temperatures were too warm for proper growth (although exact temperatures were not measured) (Meynet, 1993). Subsequent experiments were scheduled to avoid storage treatments ending during an unfavorable season. In Ithaca, NY, *R. asiaticus* plants grow best in the greenhouse from late fall through early spring, when temperatures can more reliably be kept below ≈20 °C. This observation is consistent with common cultural recommendations for growth (Meynet, 1993).

Because *R. asiaticus* is native to a desert climate, it is not surprising that relatively dry handling is superior to a humidified environment. In our experiments, TRs stored at 60% relative humidity or below maintained viability over 16 to 20 weeks when stored at 5 or 20 °C. For commercial handlers, low temperature should be the first priority when storing *R. asiaticus* TRs, but attention should be given to keep temperatures above freezing when humidity is not controlled. Sakai (1960) found *R. asiaticus* TRs survived submersion in liquid nitrogen for 24 h in the dry state, but minimal hydration resulted in viability loss at –5 °C. Based on our findings, a combination of low temperature and low humidity provided the highest *R. asiaticus* TR viability and should therefore be considered optimum for long-term storage.

Literature Cited

Beruto, M., M. Fibiani, S. Rinino, R. Lo Scalzo, and P. Curir. 2009. Plant development of *Ranunculus asiaticus* L. tuberous roots is affected by different temperature and oxygen conditions during storage period. Isr. J. Plant Sci. 57:377–388.

Bewley, J.D. and M. Black. 1994. Seeds: Physiology of development and germination. 2nd Ed. Plenum Press, New York, NY.

Copeland, L.O. and M.B. McDonald. 2001. Principles of seed science and technology. 4th Ed. Kluwer Academic Publishers, Boston, MA.

De Hertogh, A.A. 1996. Holland bulb forcer's guide. 5th Ed. Alkemade Printing BV, Lisse, The Netherlands.

Forney, C.F. and D.G. Brandl. 1992. Technology and product reports: Control of humidity in small controlled environment chambers. HortTechnology 2:52–54.

Hopkins, W.G. 1999. Introduction to plant physiology. 2nd Ed. John Wiley and Sons, Inc., New York, NY.

Justice, O.L. and L.N. Bass. 1978. Principles and practices of seed storage. U.S. Dept. Agr. Hdbk. 506:1–289.

Meynet, J. 1993. Ranunculus, p. 603–610. In: De Hertogh, A.A. and M. Le Nard (eds.). Physiology of flower bulbs. Elsevier Science Publishers, Amsterdam, The Netherlands.

Ozga, J.A., D.M. Reinecke, N.R. Knowles, and P. Blenis. 2004. Characterization of the loss of seedling vigor in pea (*Pisum sativum* L.). Can. J. Plant Sci. 84:443–451.

Priestley, D.A. 1986. Seed aging: Implications for seed storage and persistence in the soil. Comstock Publishing Associates, Ithaca, NY.

Sakai, A. 1960. The frost-hardiness of bulbs and tubers. J. Hort. Assoc. Japan 29:233–238 [Engei Gakkai Zasshi].