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# Comparison of Dry Matter Loss Rates from Static and Dynamic Grain Respiration Measurement Systems for Soybeans at 18% Moisture Content and 30°C

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## Abstract

Time to reach 0.5% dry matter loss (DML) is the estimated maximum allowable storage time (MAST) for shelled corn and has been suggested for use with other grains. Respiration studies have reported various estimates of this threshold depending on the type of grain respiration measurement system (GRMS) and storage conditions tested. The objectives of this study were (1) to design and evaluate two GRMS in which oxygen needed for respiration was limited in a static system (S-GRMS) or continuously supplied in a dynamic system (D-GRMS) during storage and (2) to compare the effects of GRMS on DML rates (vDML) for 18% moisture content soybeans stored at 30°C for 20 d. In this study, S-GRMS and D-GRMS units were designed to conduct respiration tests. Respired CO2 (mg CO2) was measured over time and used to calculate the specific mass of respired CO2 (mg CO2 kg-1 d.b. beans) and subsequent DML (%) using stoichiometric ratios from the respiration chemical reaction. DML rates, vDML (% d-1), were estimated by least squares linear regression of DML and time data. Four replications of respiration tests were conducted in each GRMS. Average estimates of vDML were 0.0157% d-1 and 0.0189% d-1 for S-GRMS and D-GRMS, respectively. Mean vDML from D-GRMS tests was 1.2 times greater than mean vDML from S-GRMS but not statistically different (p = 0.09). However, the coefficient of variation was 8 times greater for D-GRMS than for S-GRMS. More studies with a wider range of storage conditions should be conducted for development of a safety factor between both systems prior to using data from respiration of soybeans in the literature to estimate MAST.



## Keywords

Dry matter loss, Grain storage, Respiration, Soybeans


## Disciplines

Agriculture | Bioresource and Agricultural Engineering

## Comments


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# Comparison of Dry Matter Loss Rates from Static and Dynamic Grain Respiration Measurement Systems for Soybeans at 18% Moisture Content and 30°C

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## Highlights

- Design, description, and comparison of static (S) and dynamic (D) grain respiration measurement systems (GRMS).
- No differences were detected between dry matter loss rates ($v_{DML}$) from S-GRMS and D-GRMS for soybeans at 18% moisture content and 30°C stored for 20 d.
- Literature reports variable $v_{DML}$ estimates for soybeans stored in S-GRMS and D-GRMS; more studies should be conducted with a wider range of storage conditions before developing maximum allowable safe storage time guidelines.

**Abstract.** *Time to reach 0.5% dry matter loss (DML) is the estimated maximum allowable storage time (MAST) for shelled corn and has been suggested for use with other grains. Respiration studies have reported various estimates of this threshold depending on the type of grain respiration measurement system (GRMS) and storage conditions tested. The objectives of this study were (1) to design and evaluate two GRMS in which oxygen needed for respiration was limited in a static system (S-GRMS) or continuously supplied in a dynamic system (D-GRMS) during storage and (2) to compare the effects of GRMS on DML rates ($v_{DML}$) for 18% moisture content soybeans stored at 30°C for 20 d. In this study, S-GRMS and D-GRMS units were designed to conduct respiration tests. Respired $CO_2$ (mg $CO_2$) was measured over time and used to calculate the specific mass of respired $CO_2$ (mg $CO_2$ kg$^{-1}$ d.b. beans) and subsequent DML (%) using stoichiometric ratios from the respiration chemical reaction. DML rates, $v_{DML}$ (% d$^{-1}$), were estimated by least squares linear regression of DML and time data. Four replications of respiration tests were conducted in each GRMS. Average estimates of $v_{DML}$ were 0.0157% d$^{-1}$ and 0.0189% d$^{-1}$ for S-GRMS and D-GRMS, respectively. Mean $v_{DML}$ from D-GRMS tests was 1.2 times greater than mean $v_{DML}$ from S-GRMS but not statistically different ($p = 0.09$). However, the coefficient of variation was 8 times greater for D-GRMS than for S-GRMS. More studies with a wider range of storage conditions should be conducted for development of a safety factor between both systems prior to using data from respiration of soybeans in the literature to estimate MAST.*

**Keywords.** *Dry matter loss, Grain storage, Respiration, Soybeans.*

World oilseed production in 2018-2019 was 596 million metric tons, of which 358 million metric tons were soybeans (USDA, 2020). A significant amount of grain that is produced can be lost during harvesting and handling. Furthermore, if grain is not managed properly during storage, its quality declines during storage. For example, soybean post-harvest loss in Brazil was estimated at 2.7% during storage (Grolleaud, 2002). Grain loss during storage may be due to spoilage organisms, such as insects, mites, rodents, and molds, which develop more rapidly when there are unfavorable storage conditions, such as high moisture content and temperature (Coker, 1994). Even without spoilage and under favorable storage conditions, grains continue to respire and lose dry matter. Therefore, to determine the maximum allowable storage time (MAST) of grain, it is important to have a method to measure and predict the rate of dry matter loss (DML) under a range of storage conditions.

Respiration is an oxidative reaction of glucose ($C_6H_{12}O_6$), with adenosine diphosphate (ADP) and inorganic phosphate ($P_i$), into carbon dioxide ($CO_2$) and water ($H_2O$) that produces energy in the form of adenosine triphosphate (ATP) and kilocalories (kcal):

$$C_6H_{12}O_6 + 6O_2 + 38ADP + 38P_i \rightarrow 6CO_2 + 6H_2O + 38ATP + 686\ \text{kcal} \quad (1)$$

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Equation 1 shows the relationship between the respiration process and substrate losses. For every mole of respired $C_6H_{12}O_6$ (180 g mol$^{-1}$), 6 moles of $CO_2$ (6 moles × 44 g mol$^{-1}$) are produced while 6 moles of $O_2$ (6 moles × 16 g mol$^{-1}$) are consumed. Monitoring respired $CO_2$ during grain storage is one method used to detect activity of mold in stored grain so that appropriate interventions can be made to restrain or stop fungal growth (Huang et al., 2013). ASABE Standard D535 (ASABE, 2019) uses 0.5% DML as the threshold to determine MAST of shelled corn, equivalent to 7.33 g of $CO_2$ per kg of dry matter.

Respiration rates will change depending on many factors. Intrinsic factors include the grain type and genotype, its development phase at harvest, chemical composition, and moisture content. Extrinsic factors include temperature, concentrations of $O_2$ and $CO_2$, hydrocarbons, and stresses, such as biological stresses caused by incidence of disease (Kader and Saltveit, 2002a). As with most chemical reactions, respiration increases with increasing temperature. Higher respiration rates reduce the post-harvest life of a commodity. Respiration has been expressed in terms of decreased $O_2$ levels ($m_{O2,s}$, mg $O_2$ kg$^{-1}$ dry matter), increased levels of respired $CO_2$ ($m_{CO2,s}$, mg $CO_2$ kg dry matter$^{-1}$), or DML (%).

Since the 1940s, grain quality degradation research has been conducted based on measurement of respired $CO_2$ that was used to calculate DML by means of equation 1. Several studies are summarized in table 1 by grain commodity, $CO_2$ measurement method, moisture content and temperature during storage, and deterioration. In general, respiration rates are greater at higher temperatures and moisture contents. The results in table 1 are discussed in more detail later in this section.

Respired $CO_2$ is typically measured using either static or dynamic grain respiration measurement systems (S-GRMS or D-GRMS, fig. 1). The difference between these two systems is the availability of air, specifically $O_2$, during the grain respiration tests. Kader and Saltveit (2002b) stated that, for fruits and vegetables, elevated levels of $CO_2$ above 10% can decrease respiration rates, compositional changes, and deterioration. A D-GRMS could be used to simulate aerated bulk storage, while S-GRMS could simulate non-aerated storage, such as barges, ships, and rail cars.

In S-GRMS (fig. 1a), grain is placed in a sealed chamber and $O_2$ is depleted while products of respiration ($CO_2$ and water vapor) accumulate. The chamber is hermetically sealed, and accurate measurement of the $CO_2$ concentration ($C_{CO2}$) can be made with a gas chromatograph, infrared $CO_2$ analyzer, gas pressure sensor, or $CO_2$ absorbent material. Saltveit (2019) described the measurement process for respiration rate ($v_{CO2}$) based on monitoring $C_{CO2}$ in S-GRMS

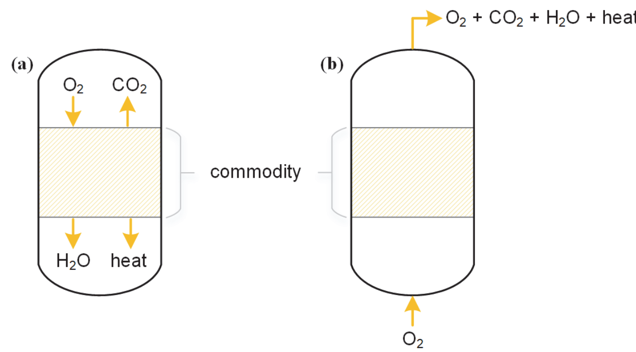


**Figure 1. Schematic of (a) static and (b) dynamic grain respiration measurement systems (S-GRMS and D-GRMS).**

**Table 1. Measurement methods and ranges of corn and soybean respiration rates reported at various storage conditions.**

| Grain Commodity | $CO_2$ Measurement Used in GRMS[a] | | Storage Conditions[b] $w$ (% w.b.) | Storage Conditions[b] $T$ (°C) | Grain Deterioration $v_{CO2}$ (mg kg$^{-1}$ h$^{-1}$) | Grain Deterioration $t_{0.5}$ (d) | Grain Deterioration $v_{DML}$ (× 10$^{-3}$ % d$^{-1}$) | Reference |
|---|---|---|---|---|---|---|---|---|
| Corn | D | Abs | 19 to 28 | 18 | 9 to 100 | 5 to 38[c] | 13 to 108[d] | Steele (1967) |
| | D | Abs | 19 to 22 | 26 | 13 to 50 | 6 to 25[c] | 20 to 80[d] | Fernandez et al. (1985) |
| | D | Abs | 21 | 26 | 20 to 27 | 12 to 15[c] | 33 to 44[d] | Friday et al. (1989)[f] |
| | D | Abs | 22 | 15 to 25 | - | 11 to 14[c] | 36 to 45[e] | Al-Yahya (1991) |
| | D | IR | 18 to 22 | 20 | - | 10 to 43 | 12 to 51[e] | Gupta et al. (1999) |
| | D | NDIR | 24 | 20 | - | 8 to 9[c] | 54 to 66[e] | Wilcke et al. (2001) |
| | S | GC | 14 to 22 | 30 | - | 15 to 55 | 9 to 33[e] | Weinberg et al. (2008) |
| | S | NDIR | 14 to 22 | 10 to 30 | 10 to 45 | - | 16 to 74[d] | Huang et al. (2013) |
| | S | PS | 13 to 21 | 23 to 45 | 0 to 48 | - | 0 to 78[d] | Ubhi and Sadaka (2015) |
| Soybean | D | Abs | 17 to 25 | 25 to 41 | 5 to 46 | - | 8 to 75[d] | Ramstad and Geddes (1942) |
| | D | GC | 14 to 26 | 15 to 30 | - | 7 to 47 | 10 to 70[e] | Sorour and Uchino (2004) |
| | D | Abs | 9 to 21 | 26 | - | 10 to 26 | 19 to 50[e] | Rukunudin et al. (2004) |
| | S | GC | 23 | 15 to 35 | 1.90 to 11.21 | - | 3 to 20[d] | Jian et al. (2014) |
| | S | DM | 13 | 21 | - | 64 to 111 | 4.5 to 7.8[e] | Hartmann Filho et al. (2016)[g] |

[a] Grain respiration measurement systems (GRMS) were either static (S) or dynamic (D) and used different instruments to measure respired $CO_2$: gravimetric using $CO_2$ absorbent material (Abs), infrared spectrophotometer (IR), nondispersive infrared analyzer (NDIR), gas chromatography (GC), pressure sensor (PS), or dry matter mass (DM).

[b] Grain moisture content ($w$) and storage temperature ($T$).

[c] Values have been converted to hourly respiration rate or to days for comparison.

[d] Grain deterioration reported as respiration rate ($v_{CO2}$). Values for dry matter loss rate ($v_{DML}$) were estimated using the following equation:

$$v_{DML} = \left[ \left( M_{C_6H_{12}O_6} / 6\, M_{CO_2} \right) v_{CO_2} \left( 24 \text{ h/d} \right) 10^{-4} \right]$$

[e] Grain deterioration reported as time to reach 0.5% DML ($t_{0.5}$). Values for dry matter loss rate ($v_{DML}$) were estimated using the following equation:

$$v_{DML} = \left( 0.5\% \text{ DML} / t_{0.5} \right)$$

[f] At a single set of grain $w$ and storage $T$, grain deterioration was reported for different corn hybrids.

[g] Grain deterioration reported based on sets of grain dried at five different $T$ (40°C to 80°C in 10°C steps) to achieve the same $w$, and then stored at the same $T$ = 21.4°C.

and D-GRMS. For S-GRMS, $v_{CO_2}$ can be mathematically described as:

$$v_{CO_2} = \frac{dm_{CO_2,s}}{dt} = \frac{\Sigma m_{CO_2,V}}{\Delta t}\frac{V_c}{m_{dm}} \quad (2)$$

where $dm_{CO_2,s}/dt$ is the change in specific mass of $CO_2$ over time (mg $CO_2$ $kg^{-1}$ dry matter $h^{-1}$), $\Sigma m_{CO_2,V}$ is the accumulated mass of respired $CO_2$ per unit volume in the chamber (mg $CO_2$ $m^{-3}$), $V_c$ is the container volume ($m^3$), $\Delta t$ is the duration of time between the collected samples of $C_{CO_2}$ (h), and $m_{dm}$ is the dry matter of the grain (e.g., kg dry beans).

While S-GRMS are easy to set up, the system does not reach equilibrium because the $O_2$ concentration is reduced while $m_{CO_2,s}$ accumulates with other gases (e.g., $H_2O$ vapor), and the temperature increases as a result of the exothermic respiration process, thereby influencing $v_{CO_2}$ measurements (Kader and Saltveit, 2002a). Lacey et al. (1994) stated that an increase in $C_{CO_2}$ can inhibit aerobic respiration and enable other respiration pathways, including anaerobic. Thus, these systems typically run for shorter periods, and the magnitude of $v_{CO_2}$ will be affected by the hermetic seal quality, relative mass of respiring grain, and accuracy of the instrumentation.

Some researchers have devised ways to replenish $O_2$ inside the chamber during testing so that, strictly speaking, their results are not from an S-GRMS. For example, White et al. (1982) opened the grain-filled flask after each gas sampling and flushed the flask with air for 2 to 5 min. Lacey et al. (1994) used a laboratory electrolytic respirometer developed by Tribe and Maynard (1988) that enabled $O_2$ to be replenished over time inside a sealed chamber. As $O_2$ was consumed by the grain, respired $CO_2$ was absorbed by an alkali solution. The decrease in gas pressure triggered an electrode to contact an anode, and $O_2$ was generated at the anode as copper was deposited on the cathode.

In contrast, in a D-GRMS (fig. 1b), conditioned air flows continuously through the grain, delivering a constant supply of $O_2$ and extracting respiration products (Saltveit, 2019). Likewise, $v_{CO_2}$ can be mathematically described by:

$$v_{CO_2} = \frac{dm_{CO_2,s}}{dt} = \frac{\Sigma m_{CO_2,V}}{\Delta t} Q \quad (3)$$

where $Q$ is the flow rate of air through the system (L $h^{-1}$).

In this system, the accuracy of $v_{CO_2}$ will depend on controlling $Q$. Such a system can be run for extended periods, and the gas mixture of the air supply can be conditioned to maintain constant temperature, relative humidity, and $O_2$ levels during the respiration test. Operating a D-GRMS requires continuous monitoring of input and output airflow conditions with feedback into a control system so that equilibrium conditions are maintained during testing (Kader and Saltveit, 2002a).

Alternatively, it is also possible to measure $v_{CO_2}$ from a dynamic system using $CO_2$ absorption. In this case:

$$v_{CO_2} = \frac{dm_{CO_2,s}}{dt} = \frac{\Sigma m_{CO_2}}{\Delta t\left(m_{dm}\right)} \quad (4)$$

where $\Sigma m_{CO_2}$ is the absorbed mass of $CO_2$ (mg $CO_2$). This method is a direct gravimetric option that eliminates the need for precise $Q$ measurement. Such a system was documented by Rukunudin (1997), Sood (2015), and Trevisan (2017), and most studies that measured respired $CO_2$ used a D-GRMS (table 1).

In the literature, grain deterioration has been defined in terms of $v_{CO_2}$ or dry matter loss rate ($v_{DML}$). Another metric used is the elapsed time to reach 0.5% DML ($t_{0.5}$) as a quality indicator of grain storability. Steele (1967) proposed using time to achieve a 0.5% DML threshold as the maximum time to store shelled corn because he observed that this threshold coincided with corn losing one grade level (e.g., from U.S. Standard Grade No. 1 to No. 2). Rukunudin (1997) analyzed total damaged seeds on preserved soybeans and found a similar grade reduction at $t_{0.5}$.

A wide range of $v_{CO_2}$, $v_{DML}$, and $t_{0.5}$ measurements is reported in the literature, despite similarities in grain storage conditions (table 1). For example, shelled corn with 21% to 22% moisture content stored at 25°C reached 0.5% DML in 12 to 15 d, according to Friday et al. (1989) and Al-Yahya (1991), who used a D-GRMS in their studies. However, in another study, 21% moisture content corn stored at 23°C for 9 d in an S-GRMS reached 0.00062% DML (Ubhi and Sadaka, 2015). For soybeans, Sorour and Uchino (2004) achieved 0.5% DML after 22 d in a D-GRMS for 22% moisture content beans at 25°C, and Rukunudin et al. (2004) reached the same point in 12 d for 21% moisture content beans at 26°C. Yet, like Ubhi and Sadaka (2015), Jian et al. (2014) did not achieve $t_{0.5}$; after 30 d, the maximum DML was 0.00064% in an S-GRMS for 23% moisture content soybeans at 25°C.

The discrepancies in reported DML in table 1 may be due to differences in intrinsic factors or to the GRMS used. Therefore, the objectives of this study were (1) to design and evaluate two GRMS in which oxygen needed for respiration was limited in S-GRMS or continuously supplied in D-GRMS throughout storage and (2) to compare effects of GRMS on $v_{DML}$ estimates for 18% moisture content soybeans stored at 30°C for 20 d. Storage conditions chosen in this study are typical for soybean harvest and initial storage conditions in Mato Grosso, Brazil, which can range from 10.8% to 25.7% moisture content and where average ambient temperatures are 19.7°C to 35.1°C (Danao et al., 2015).

# Materials and Methods

## Preparation of Soybeans Samples

Soybeans (P35T75X RR2X, DuPont Pioneer, Johnston, Iowa) were combine harvested at 11.1% moisture content (w.b.) from the Crop Sciences Research and Education Farm of the University of Illinois at Urbana-Champaign on September 29, 2017. The beans were stored in plastic containers (68 L and 50 kg capacity) at 4°C until sample preparation (fig. 2) at the start of each respiration test.

Before the start of each test, soybeans were mixed manually in a container, and a 3 kg sample ($m_{soy,0}$) was retrieved and cleaned using a sieve (10/64 in. × 3/4 in., Grainman, Miami, Fla.) to remove impurities and splits or damaged beans.

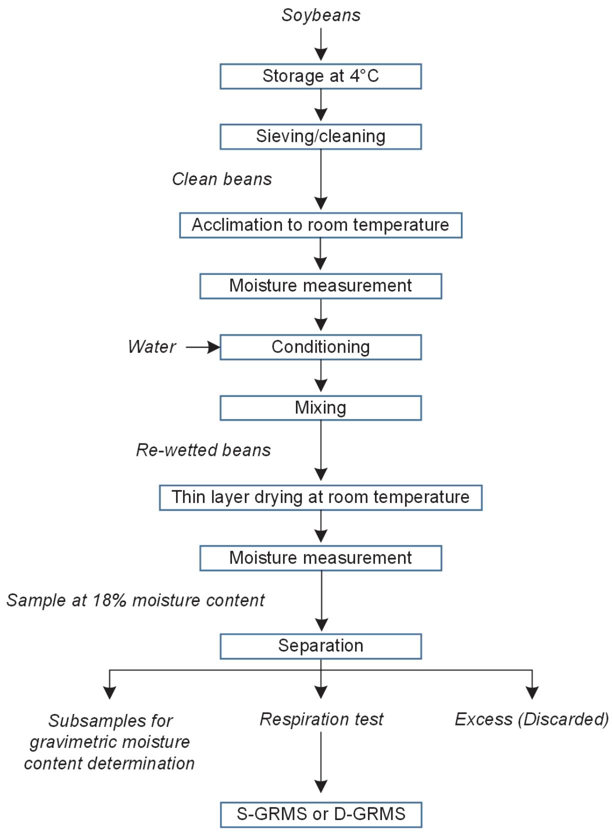


**Figure 2. Soybean sample preparation for S-GRMS and D-GRMS.**

The sample was acclimated at room temperature (22°C to 23°C) for 30 to 40 min. The initial moisture content $\left(\hat{w}_{soy,0}\right)$ was estimated using a portable moisture meter (model SW16060, John Deere, Moline, Ill.) and used to calculate the amount of deionized water ($m_{H_2O}$) to be added to achieve the desired test moisture content ( $\hat{w}_{soy,1}$ = 18%):

$$m_{H_2O}\ (\%) = m_{soy,0}\left(\frac{\hat{w}_{soy,1} - \hat{w}_{soy,0}}{1 - \hat{w}_{soy,1}}\right) \times 100 \qquad (5)$$

All moisture contents reported in this study are on a wet basis (w.b.).

Soybeans were poured into two 2 L capacity plastic bottles and placed in a roller mixer (model MX-T6-S, Scilogex, Rocky Hill, Conn.) operating at 60 rpm for 60 min. After every 5 min of mixing, small aliquots of approximately 15 mL of deionized water were added until $m_{H_2O}$ was reached (83.85 to 105.20 g). Slow rotation and addition of small aliquots avoided sudden swelling of the beans, as described by Ramstad and Geddes (1942) when all the water was added at once. In addition to $m_{H_2O}$, an additional 10 mL of water was added to each container during the re-wetting process to ensure complete hydration of the beans. The re-wetted soybean sample was poured as a thin layer onto a metal tray, and excess moisture was allowed to evaporate at room temperature for 20 to 30 min. Every 5 min, $\hat{w}_{soy,1}$ was tested, and air drying ceased when 18% was reached. The actual test moisture content ($w_{soy,1}$) of each sample was determined using the 72 h, 103°C oven method according to ASABE Standard S352.2 (ASABE, 2017), with triplicate containers for each measurement.

Each rewetted sample was placed in the respective GRMS. The 3 kg of cleaned soybeans were used to set up four replications in an S-GRMS or one replication in a D-GRMS due to the difference between the storage capacities of each respiration chamber (RC). At the end of each respiration test, the moisture content was again measured using the oven method. The initial and final moisture content measurements of each test were within ±1%, and the relative humidity of the systems (88% RH ±5%) corresponded to the desired equilibrium moisture content.

## Respiration Tests

Four replications of respiration tests were conducted in each S-GRMS and D-GRMS. Both systems used 18% moisture content soybean samples at a controlled temperature of 30°C. The RC in the S-GRMS held 500 g soybeans at 18% moisture content, while the RC of the D-GRMS held 1800 g soybeans.

### *Static Grain Respiration Measurement System*

An S-GRMS (fig. 3) was set up using a hermetically sealed RC and a $CO_2$ sensor package with an internal data logger and battery pack (Catalog No. K33-BLG, CO2Meter, Inc., Ormond Beach, Fla.) placed on top of the grain. The RC was a 10 L glass desiccator, and the sensor package monitored $C_{CO_2}$ (% or v/v), temperature (°C), and relative humidity (% RH). The RC was placed in a temperature-controlled incubator (model 3033, Steri-Culti 200, Forma Scientific, Inc., Marietta, Ohio), which was preheated to 30°C. The incubator held up to four S-GRMS units.

Prior to the experiment, four sensor packages were calibrated for $C_{CO_2}$ measurements using certified gases: 100% nitrogen (0% $CO_2$) and $CO_2$-nitrogen mixtures of 0.15%, 0.5%, 1%, 5%, and 10% $CO_2$ v/v (Airgas, Inc., Danville, Ill.), the sensor manufacturer's Data Acquisition Software (DAS, CO2Meter, Inc., Ormond Beach, Fla.), and a SenseAir cable with UART communication protocol. Each reference gas was certified as ±0.03% of the labeled value

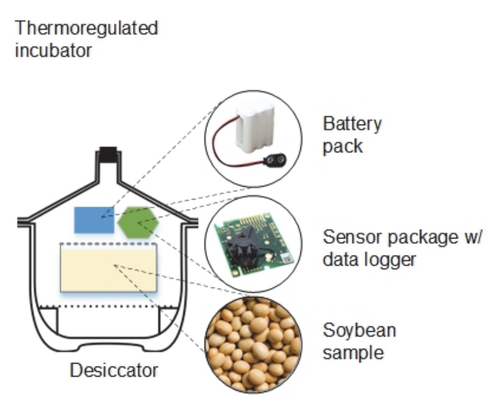


**Figure 3. Each static grain respiration measurement system (S-GRMS) included a 10 L desiccator as the respiration chamber capable of holding a 500 g soybean sample and a battery-operated sensor package to monitor temperature, relative humidity, and $C_{CO_2}$ inside the chamber. The temperature of the S-GRMS unit was controlled over time by placing it inside a thermoregulated incubator.**

according to each cylinder calibration sheet. During calibration, four sensor packages were placed in a sealed plastic bag (4 L capacity), into which the desired reference gas was introduced at 1 L min$^{-1}$ for 20 min. The sensors were set to record $C_{CO_2}$ every 20 s. Measurements stabilized after about 5 min of introducing the reference $CO_2$ gas. Henceforth, after 15 min, measurements were averaged ($\overline{C}_{CO_2}$) and regressed against the $C_{CO_2}$ reference values (fig. 4). Linear regressions were obtained from the Data Analysis ToolPak in MS Excel (ver. 2016, Microsoft, Redmond, Wash.) and used to correct respired $C_{CO_2}$ measurements. Additional information regarding the S-GRMS, test protocols, data analyses, and calibration are detailed by Pereira Da Silva (2018).

Because the incubator was large enough to accommodate four RCs, static respiration tests with four replications were conducted simultaneously using four S-GRMS units. The sensors were set to record $C_{CO_2}$ every 10 min ($C_{CO_2,t}$). The desiccator lids were sealed with vacuum grease, and the S-GRMS units were placed inside the incubator at 30°C for 20 d.

The beginning of each respiration test ($t_0$) was defined as the time when the temperature inside the desiccator reached 30°C (±1°C). For any time during the test, accumulated respired $C_{CO_2,t}$ measurements were corrected, first using the corresponding calibration equation obtained by inverting the regression equation, and then by subtracting the average gas concentration before the start of a respiration test. The ideal gas law was used to convert this adjusted concentration measurement to a specific mass:

$$\sum m_{CO_2,s} = \frac{\left(C_{CO_2,t}\right)}{m_{dm}}\left(\frac{PV\left(M_{CO_2}\right)}{RT}\right) \quad (6)$$

where $\Sigma m_{CO_2,s}$ is the accumulated specific mass of respired $CO_2$ per unit mass of dry matter of soybeans ($m_{dm}$), $P$ is the pressure (1 atm), $V$ is the air volume (RC volume minus volume of grain and volume occupied by the sensor), $R$ is the ideal gas constant (0.08205 L atm K$^{-1}$ mol$^{-1}$), $T$ is the temperature (K), and $M_{CO2}$ the molar mass of $CO_2$ (44 g mol$^{-1}$).

### *Dynamic Grain Respiration Measurement System*

Two D-GRMS units were designed and operated simultaneously to conduct respiration tests, based on the design and test protocols of Sood (2015) and Trevisan (2017). Dynamic respiration tests with four replications were conducted using two D-GRMS, two tests per unit. Each D-GRMS (fig. 5) was divided into four sections: (A) air conditioning and flow management, (B) grain storage, (C) moisture and $CO_2$ absorption columns, and (D) instrumentation. A full description of this D-GRMS is provided by Trevisan (2017), and updated test protocols are provided by Pereira Da Silva (2018).

*Section A: Air Conditioning and Flow Management*

Each D-GRMS was supplied with a mixture of compressed air (80% $N_2$, 20% $O_2$, with $C_{CO_2}$ of 400 ppm) maintained at 15 psi by a gas guard (model 3050, Forma Scientific, Inc., Marietta, Ohio). The air supply was controlled at 0.5 L min$^{-1}$ by a precision mass flow controller (model GFC17A, Aalborg, Orangeburg, N.Y., accuracy ±0.02 L min$^{-1}$). $CO_2$ present in the supplied air was removed by a scrubber containing 300 g $CO_2$ absorbent (Sodasorb, Amron Intl., Vista, Cal.) and conditioned to the desired test temperature (30°C) and equilibrium relative humidity ($\phi_e$, 88% RH) by bubbling it through a series of two 2 L plastic vacuum bottles (Catalog No. D1069702, U.S. Plastics, Lima, Ohio) of temperature-controlled glycerol-water solution (38.4% m/m), prepared following guidelines provided by Forney and Brandl (1992) for 18% (w.b.) moisture content soybeans at 30°C. The air supply $CO_2$ scrubber and vacuum bottles were placed into the system using quick-connect couplings (Catalog Nos. 60774 and 60779, U.S. Plastics, Lima, Ohio).

*Section B: Grain Storage*

The conditioned airstream passed through the grain-filled RC, which was wrapped in an insulated water jacket maintained at a temperature of 30°C. The external water jacket was made of Vincon Flexible PVC tubing (Part No. ABH02017, Saint-Gobain, Akron, Ohio) wrapped around

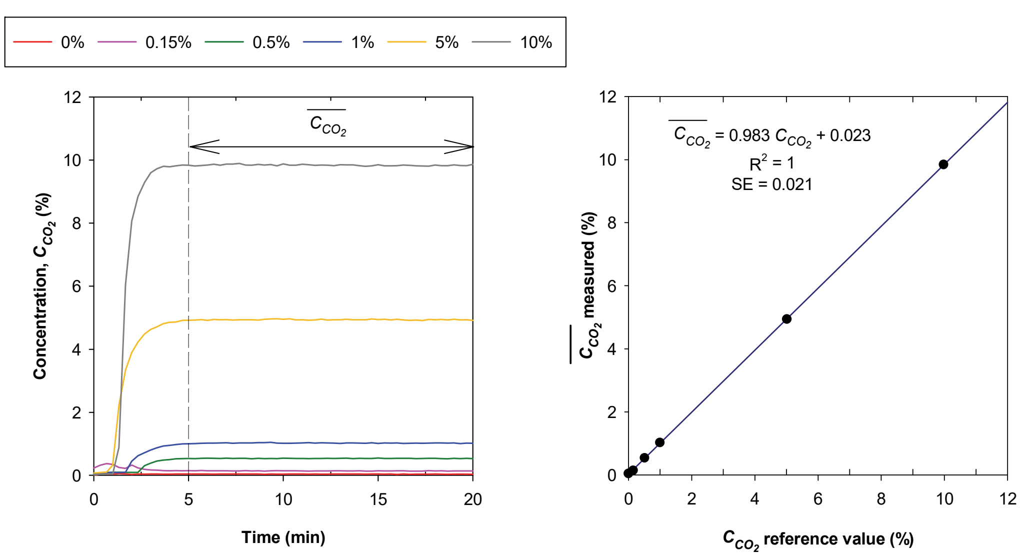


**Figure 4. Carbon dioxide concentrations over time and an example regression for one sensor package.**

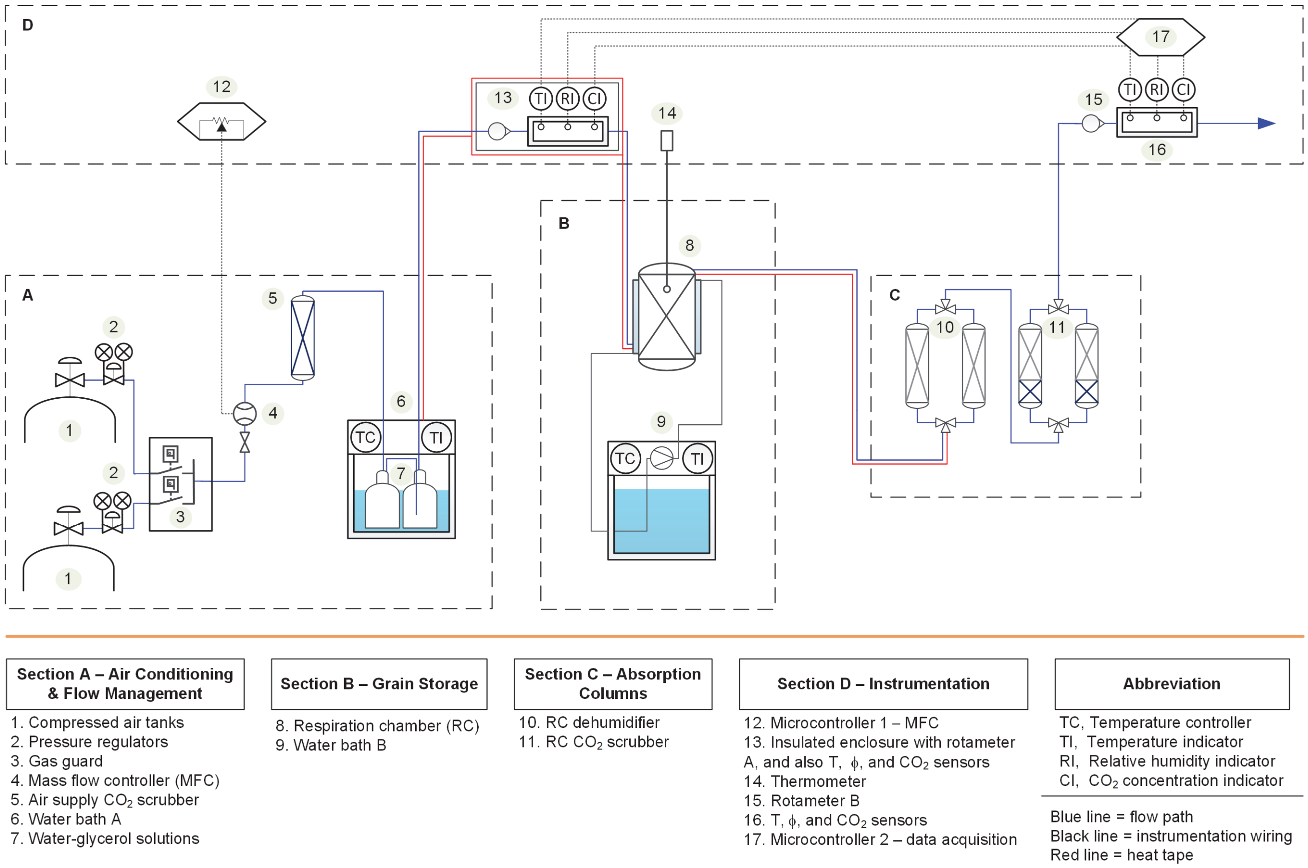


**Figure 5. Schematic of dynamic grain respiration measurement system (D-GRMS). Heat was applied to the flow tubes in section B using a 19.6 W m$^{-1}$ heating tape to prevent condensation of the humidified or respired air streams.**

the RC and was provided with a continuous flow of water at 30°C from a second water bath. Air exiting the top of the RC carried the humidified air and grain respiration products ($CO_2$ and $H_2O$ vapor). The grain storage temperature was visually monitored using a digital thermometer (model 11050, DeltaTRAK, Pleasanton, Cal.) located at the top of the RC and inserted 7.5 cm deep into the grain bed. To minimize temperature fluctuations and condensation of the incoming and exiting air streams, the RC was thermally insulated with pre-slit fiberglass pipe insulation (1 in.) and pre-slit polyethylene pipe insulation (6.35 mm thick). All tubing from the first water bath to the absorption columns was wrapped with heat tape (model W51-6p, Raychem, Houston, Tex.) to prevent surface condensation.

*Section C: Moisture and $CO_2$ Absorption Columns*

The air exiting the RC was first dehumidified using a desiccant column filled with a 500 g bed of desiccant and indicating 4-mesh desiccant (Catalog Nos. 26800 and 23025, W.A. Hammond Drierite Co., Xenia, Ohio) to remove excess moisture from humidification and grain respiration. The color indicator desiccant (i.e., blue when dry, pink when wet) allowed visual monitoring of the moisture removal process. After dehumidification, air exited the desiccant column, and respired $CO_2$ was captured using an RC $CO_2$ scrubber made of a layer of 150 g of $CO_2$ absorbent (Sodasorb, Amron Intl., Vista, Cal.) followed by 300 g of 4-mesh desiccant (Catalog No. 21001, W.A. Hammond Drierite Co.). The two layers of materials were contained in a cylinder (Catalog No. 23025, W.A. Hammond Drierite Co.) and separated by a small plastic cylinder (2.5 cm i.d. × 1.5 cm height) with perforated disks at each end (40% open, 0.3 cm dia. holes) to mitigate moisture diffusion from the $CO_2$ absorbent into the desiccant. The RC $CO_2$ scrubber and dehumidifier were initially weighed and connected to the system using quick-connect couplings (Catalog Nos. 60774 and 60779, U.S. Plastics, Lima, Ohio).

The exhaust stream from the grain respiration chamber was connected to a three-way valve for sequential distribution to one of two adsorption columns (RC $CO_2$ scrubbers). To measure $\Sigma m_{CO_2}$, the test was started using one airflow path until the first measurement, typically after a period of 12 to 14 h. The airflow was then redirected to the second RC $CO_2$ scrubber, and the scrubber with accumulated mass was removed using the quick-connect couplings, weighed, and replaced into the D-GRMS. Respired $CO_2$ was allowed to accumulate on the second RC $CO_2$ scrubber for 2 h until the airflow was diverted back to the first scrubber. Weight measurements from both sides increased over time as respired $CO_2$ was absorbed. The elapsed time for each measurement interval was recorded to the nearest minute. At any time, $\Sigma m_{CO_2}$ was the sum of the total accumulated mass of respired $CO_2$ from both scrubbers. Measurements were taken five times per day (daytime hours), approximately every 2 h for 20 d. No measurements were taken overnight. At the end of a respiration test, $\Sigma m_{CO_2}$ was normalized to $m_{dm}$ to yield $\Sigma m_{CO_2,s}$.

*Section D: Instrumentation*

The system was instrumented before and after the RC to monitor airflow, temperature ($T$), relative humidity ($\phi$), and $C_{CO_2}$. The sensors for $T$ and $\phi$ (model DHT11, WAVGAT, Caizhixing, China) were used to verify the test $T$ of 30°C ±2°C and $\phi$ of 88% RH for 18% moisture content soybeans placed between sections A and B and in the exhaust of the D-GRMS. $C_{CO_2}$was monitored using three $CO_2$ nondispersive infrared (NDIR) sensor probes and transmitters (models GMP222 and GMPG0N0, Vaisala, Boulder, Colo.) to verify $CO_2$ absorption, ensuring that the sensor readings remained below the measurement detection threshold (20 ppm) throughout each respiration test. All sensor readings were logged every 2 min with a computer using a microcontroller (ATmega2560, Arduino, Ivrea, Italy). Additional information regarding the circuitry and Arduino code used to log $T$, $\phi$, and $C_{CO_2}$ are provided by Pereira Da Silva (2018). The mass flow controller voltage input was adjusted using digital potentiometers connected to a second and third microcontroller. Finally, two rotameters (model MMA-4, Dwyer Instruments, Michigan City, Ind.) were placed at the outlets of sections A and C to confirm $Q$ downstream and to ensure that the D-GRMS was airtight and leak-free.

### *Conversion of Respired $CO_2$ to Dry Matter Loss Rate*

DML was estimated using stoichiometric ratios from the respiration chemical reaction (eq. 1) in which six moles of $CO_2$ were respired for every mole of $C_6H_{12}O_6$ consumed:

$$\text{DML (\%)} = \Sigma m_{CO_2,s}\left(\frac{1\ \text{mol}_{C_6H_{12}O_6}}{6\ \text{mol}_{CO_2}}\right)\left(\frac{M_{C_6H_{12}O_6}}{M_{CO_2}}\right)\times 100 \quad (7)$$

where $M_{C_6H_{12}O_6}$ is the molar mass of $C_6H_{12}O_6$ (180 g mol$^{-1}$).

The elapsed Gregorian time (MM/DD/YY hh:mm) was converted to Julian date (JD):

$$\text{JD} = (\text{YY})10^3 + D_j + \frac{\text{hh}}{24\ \text{h d}^{-1}} + \frac{\text{mm}}{1440\ \text{min d}^{-1}} \quad (8)$$

where the last two digits of the Gregorian year are multiplied by 1000 and added to the total number of days since January 1 of the same year ($D_j$) and the fraction of day.

The DML estimates from both the S-GRMS and D-GRMS showed an initial lag period before reaching a steady increase in DML. Therefore, a threshold of 0.05% DML was used to remove the lag period, i.e., data below this threshold value were not considered in subsequent analysis. The $v_{DML}$ was estimated by resetting the origin of (DML, time) from (0,0) to (0.05, $t_{0.05}$) (fig. 6), followed by least squares linear regression using the Regression option of the Data Analysis ToolPak in MS Excel (Office 365, Microsoft, Redmond, Wash.) and wherein the intercept was set to zero. The resulting slope was used as the estimate of $v_{DML}$. The summary output of the regression was:

- Regression statistics: coefficient of determination ($R^2$), standard error of the regression ($SE_{reg}$), and number of observations ($n$).
- Analysis of variance (ANOVA) table.

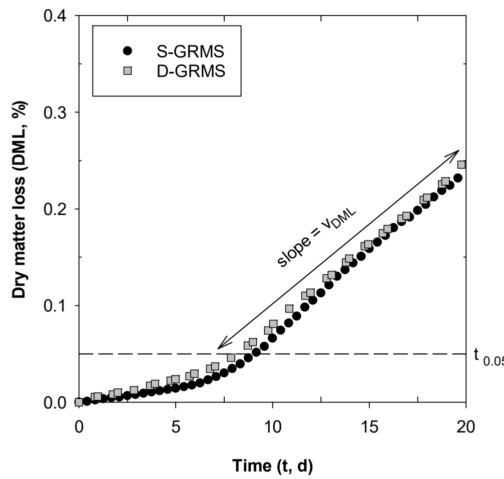


**Figure 6. Dry matter loss rates ($v_{DML}$, % d$^{-1}$) in static and dynamic grain respiration measurement systems (S-GRMS and D-GRMS) were estimated as the slope of the linear increase after DML = 0.05%. This threshold was considered the lag period of a respiration test.**

- Estimates of slope and its standard error ($v_{DML}$ ±$SE_{v_{DML}}$).
- Optional residuals and percentile plots.

## STATISTICAL ANALYSES

### *Pooled Standard Deviation*

The overall, or pooled, standard deviation of $v_{DML}$ was calculated from the mean weighted $SE_{v_{DML}}$ of each replicate slope:

$$\left(\sigma_{v_{DML}}\right)_p \cong \sqrt{\frac{\sum_{i=1}^{k}\left\{(n_i-1)\left(SE_{v_{DML}}\right)_i^2\right\}}{\sum_{i=1}^{k}(n_i-1)}} \quad (9)$$

where $n$ is the number of observations from each replicated respiration test, and $i$ denotes the replications (1, …, $k$). A total of four replications ($k$ = 4) were used for each system.

### *Comparison of Dry Matter Loss Rates*

An independent sample t-test assuming equal variance was used to compare the four replications ($n$) of $v_{DML}$ from each of the respiration systems, using the function PROC TTEST and the statements CLASS and VAR in SAS (2017 University Edition Software, SAS Institute, Inc., Cary, N.C.). The t-test was calculated based on two independent populations ($A$ and $B$), with a null hypothesis $H_0$ ($\mu_A = \mu_B$) and alternative $H_a$ ($\mu_A \neq \mu_B$), degree of freedom equal to $(n_A - 1) + (n_B - 1)$, and $\alpha = 0.05$. Populations $A$ and $B$ were the $v_{DML}$ from S-GRMS and D-GRMS, respectively.

# RESULTS AND DISCUSSION

## DRY MATTER LOSS ESTIMATES

At the start of each test, DML was relatively low for approximately 4 d in both systems and increased exponentially from day 4 to day 11 in S-GRMS and, on average, from day 5 to day 8 in D-GRMS (fig. 7). This behavior was also noted by Pereira Da Silva (2018), Trevisan (2017), and Rukunudin

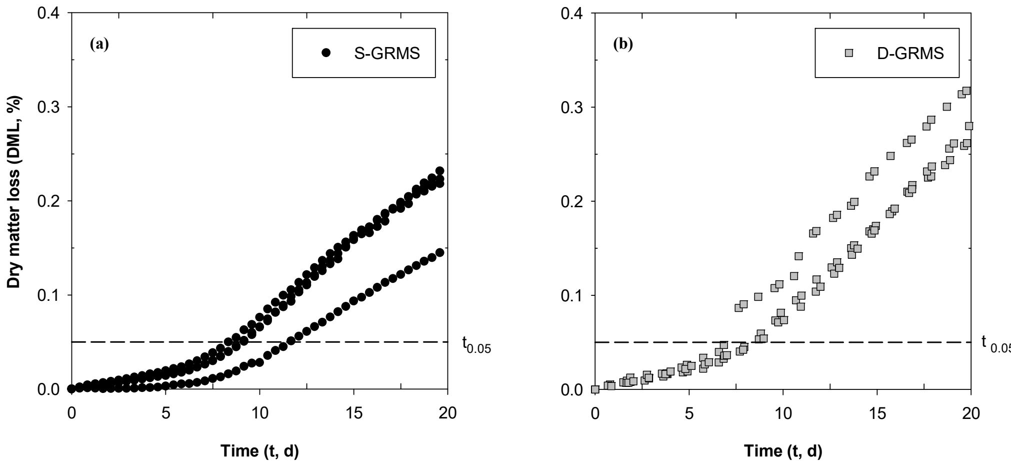


**Figure 7. Dry matter loss estimates (DML, %) over time (*t*, d) of 18% moisture content soybeans at 30°C in (a) static and (b) dynamic grain respiration measurement systems (S-GRMS and D-GRMS).**

et al. (2004); in their experiments, soybeans were stored in D-GRMS at different levels of moisture content (9% to 22%) and temperature (26°C to 35°C). However, other studies in which soybeans were stored in S-GRMS did not report relatively low DML at the beginning of their tests (Jian et al., 2014; Ochandio et al., 2012). The steady increase after day 4 to day 8 might be due to mold growth on the soybeans, which was observed by Rukunudin et al. (2004). They reported visible mycelial growth after 4 to 13 d of storage, depending on whether their soybeans were combine-harvested or hand-harvested. According to their study, combine-harvested soybeans exhibited faster visible mold growth than hand-harvested soybeans, and the dominant mold species were field fungi.

The magnitude of DML estimates was about 1.4 times higher in D-GRMS than in S-GRMS (fig. 7). The range in final DML at the end of each replicate was 0.15% to 0.23% for 18% moisture content soybeans stored in S-GRMS and 0.25% to 0.33% in D-GRMS for the same storage time of 20 d. At the end of each experiment, samples from both systems exhibited visible mold dispersed throughout the entire sample, as verified by inspection.

### Comparison of Estimated Dry Matter Loss Rates with Static and Dynamic Systems

Estimates of $v_{DML}$ (table 2) for 18% moisture content soybeans stored at 30°C ranged from 0.0123% to 0.0175% d$^{-1}$ with $\overline{v}_{DML}$ and $(\sigma_{vDML})_p$ of 0.0157% d$^{-1}$ ±0.00001% d$^{-1}$ for S-GRMS and from 0.0165% to 0.0217% d$^{-1}$ with $\overline{v}_{DML}$ and $(\sigma_{vDML})_p$ of 0.0189% d$^{-1}$ ±0.00010% d$^{-1}$ for D-GRMS. Comparing these estimates, the dynamic system had $v_{DML}$ values 1.2 times higher and $(\sigma_{vDML})_p$ 10 times greater. However, mean DML rates was not statistically different (p = 0.09).

**Table 2. Dry matter loss rates of 18% moisture soybeans stored at 30°C in static and dynamic grain respirations measurement systems.**

| Replication | Dry Matter Loss Rate and Standard Error[a] Static | Dynamic |
|---|---|---|
| 1 | 0.0175 ±0.00001 | 0.0184 ±0.00004 |
| 2 | 0.0123 ±0.00001 | 0.0217 ±0.00014 |
| 3 | 0.0168 ±0.00002 | 0.0165 ±0.00003 |
| 4 | 0.0162 ±0.00001 | 0.0190 ±0.00013 |
| Mean and pooled SD[b] | 0.0157 ±0.00001 a | 0.0189 ±0.00010 a |
| CV (%)[c] | 0.0637 | 0.5291 |

[a] $v_{DML}$ ±SE$_{vDML}$ (% d$^{-1}$). SE = standard error.

[b] $\overline{v}_{DML}$ and $(\sigma_{vDML})_p$ (% d$^{-1}$). Means followed by the same letter are not significantly different (p > 0.05). SD = standard deviation.

[c] CV = coefficient of variation.

Both systems had $v_{DML}$ values similar in magnitude to those reported by Sorour and Uchino (2004), Rukunudin et al. (2004), and Jian et al. (2014). Sorour and Uchino (2004) stored soybeans at 18% moisture content and 30°C in a D-GRMS for 30 d and had $v_{DML}$ equal to 0.0178% d$^{-1}$. Also using a dynamic system but at lower temperature (26°C) and higher moisture content (21% w.b.), Rukunudin et al. (2004) reached 0.5% DML in 26 d, which is equivalent to a $v_{DML}$ of 0.0192% d$^{-1}$. On the other hand, Jian et al. (2014) had a $v_{DML}$ estimate of 0.020% d$^{-1}$ for 23% moisture content soybeans stored in an S-GRMS at 35°C.

Because DML is directly related to respired $CO_2$, the small numerical difference measured between the systems may be explained in part by the difference in long-term availability of $O_2$ for respiration. In a static system, $O_2$ becomes limiting over time, whereas in a dynamic system, $O_2$ levels are kept constant by a continuous flow of air through the grain bed. As stated earlier, it may also be possible that elevated $CO_2$ concentrations above 10% may contribute to limiting respiration for the inhibition of mold growth (Kader and Saltveit, 2002b). Kader and Saltveit (2002b) also stated that respiration rates, compositional changes, and deterioration can be decreased by elevated levels of $CO_2$ or reduced levels of $O_2$. However, the magnitude of $v_{DML}$ for S-GRMS was not significantly lower ($\alpha$ = 0.05), which may be a confirmation that $O_2$ is not at levels which limit respiration over a 20 d test with a 500 g soybean sample. For example, in one respiration test in which $C_{CO_2}$ became approximately 8% (v/v) after 20.1 d, the $O_2$ concentration ($C_{O_2}$) would be depleted by 8% (v/v), i.e., $C_{O_2}$ would drop from 21% (v/v) to approximately 13%. This depletion may be insufficient to limit respiration, suggesting a longer-term measurement with a higher volume ratio of sample:RC or some other methods to reduce $O_2$ levels.

On the other hand, the variability of both systems should be considered. Although quite low for both systems, the coefficient of variation (CV) for D-GRMS of 0.529% was 8 times greater than the CV for S-GRMS (0.064%), suggesting that $v_{DML}$ is more variable for dynamic respiration tests than for static tests. Fewer studies in a limited range of storage temperatures and moisture contents have been conducted on soybeans than corn. Statistical analysis did not detect differences between the two systems; future comparisons should be made over a wider range of temperatures and moisture contents. When conducting tests with a static system, care must be taken to ensure that neither depletion of $O_2$ nor accumulation of $CO_2$ within the chambers inhibits microbial activity in the sample being tested. Including an $O_2$ sensor in the S-GRMS to monitor gas consumption would help confirm if 20% gas concentration was reached in the volume of air inside the respiration chamber. Therefore, the $v_{DML}$ data reported in the literature for soybeans stored in both systems should be used with caution and perhaps with a safety factor to estimate MAST until more studies with a wider range of storage conditions are conducted. Further studies that enhance either of the systems could lead to better understanding of this variability.

## CONCLUSIONS

This study compared two systems that can be used to measure respiration rates of soybeans using a limited range of storage conditions. Overall dry matter loss rate ($v_{DML}$) estimates from soybean samples at 18% moisture content stored at 30°C for 20 d were found to be about 1.20 times lower (but not different) when measured using a static grain respiration measurement system (S-GRMS) compared with a dynamic system (D-GRMS). The relatively small difference in $v_{DML}$ observed in this research using S-GRMS and D-GRMS may have been because depleted $O_2$ levels in S-GRMS were not sufficient to limit respiration, which reinforces the idea of the influence of the oxygen supply on soybean respiration rate. Mean values of $v_{DML}$ were 0.016% and 0.019% $d^{-1}$ with pooled standard deviation $(\sigma_{vDML})_p$ of $1 \times 10^{-5}$ and $1 \times 10^{-4}$ % $d^{-1}$ and coefficient of variation (CV) of 0.064% and 0.529% for S-GRMS and D-GRMS, respectively. Even though no statistical differences were detected between the mean $v_{DML}$ values for S-GRMS and D-GRMS, the mean $v_{DML}$ from D-GRMS was 8 times more variable than the mean $v_{DML}$ from S-GRMS. Care should be taken when interpreting reported $v_{DML}$ values in the literature. When $v_{DML}$ values are underestimated, the time to reach 0.5% DML ($t_{0.5}$) increases, which can lead to storage time recommendations that exceed the intended quality threshold.


### ACKNOWLEDGEMENTS

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## NOMENCLATURE

Abs = carbon dioxide absorbent material
ADP = adenosine diphosphate
ANOVA = analysis of variance
ATP = adenosine triphosphate
$C_6H_{12}O_6$ = glucose, a carbohydrate
$C_{CO_2}$ = carbon dioxide concentration (% or v/v)
$\bar{C}_{CO_2}$ = average carbon dioxide concentration (% or v/v)
$C_{CO_2,t}$ = carbon dioxide concentration over time (% or v/v)
CI = carbon dioxide concentration indicator
$C_{O_2}$ = oxygen concentration (% or v/v)
$CO_2$ = carbon dioxide
D-GRMS = dynamic grain respiration measurement system
$D_j$ = total number of days since January 1
$dm_{CO_2,s}/dt$ = specific mass change in $CO_2$ over time (mg $CO_2$ kg$^{-1}$ dry matter)
DM = dry matter
DML = dry matter loss (%)
GC = gas chromatography
$H_2O$ = water
$H_0$ = null hypothesis
$H_a$ = alternate hypothesis
hh = hour, military time (24-hour clock) (h)
IR = infrared spectrophotometer
JD = Julian date
$k$ = number of replications
$M_{C6H12O6}$ = molar mass of glucose (180 g mol$^{-1}$)
$M_{CO_2}$ = molar mass of carbon dioxide (44 g mol$^{-1}$)
$m_{CO_2,s}$ = specific mass of respired carbon dioxide (mg $CO_2$ kg$^{-1}$ dry matter)
$m_{dm}$ = mass of dry matter (kg
MFC = mass flow controller
$m_{H_2O}$ = mass of water (g)
$m_{O_2,s}$ = specific mass of consumed oxygen (mg $O_2$ kg$^{-1}$ dry matter)
$m_{soy,0}$ = mass of initial soybean sample (kg)
$m_{sub,d}$ = mass of dried subsample (kg)
$m_{sub,w}$ = mass of rewetted subsample (kg)
mm = minutes (min)
$n$ = number of observations
$N_2$ = nitrogen
NDIR = nondispersive infrared
$O_2$ = oxygen
p = probability (p-value)
$P$ = pressure
$P_i$ = inorganic phosphate
PS = pressure sensor
$Q$ = volumetric flow rate (L min$^{-1}$)
$R$ = ideal gas constant
$R^2$ = coefficient of determination that signifies goodness-of-fit of a regression model
RC = respiration chamber
RI = relative humidity indicator
S-GRMS = static grain respiration measurement system
$SE_{reg}$ = standard error of nonlinear regression; in this study, the dependent variable was dry matter loss rate (% d$^{-1}$)
$SE_{vDML}$ = standard error of dry matter loss rate (% d$^{-1}$)
$T$ = storage temperature (°C or K)
$t$ = storage time (d)

$t_0$ = start of a respiration test (d)
$t_{0.5}$ = time to reach 0.5% dry matter loss (d)
TC = temperature controller
TI = temperature indicator
$t_s$ = safe storage time (d)
$V$ = volume
$V_c$ = volume of container (L)
$v_{CO_2}$ = respiration rate (mg $CO_2$ kg$^{-1}$ h$^{-1}$)
$v_{DML}$ = dry matter loss rate (% d$^{-1}$)
$w$ = moisture content (% w.b.)
$w_{soy,1}$ = desired soybean moisture content, determined gravimetrically (% w.b.)
$\hat{w}_{soy,0}$ = estimated initial soybean moisture content, determined with moisture meter (% w.b.)
$\hat{w}_{soy,1}$ = estimated desired soybean moisture content, determined with moisture meter (% w.b.)
$w_{soy,r}$ = soybean moisture content per replicate, determined gravimetrically with three replicates (% w.b.)
$\overline{w}_{soy,r}$ = average soybean moisture content of three replicates, determined gravimetrically (% w.b.)
YY = last two digits of Gregorian year

**GREEK LETTERS**

$\alpha$ = level of statistical significance)
$\Delta t$ = time interval of collected samples (h)
$\mu$ = treatment mean or average (varies)
$(\sigma_{vDML})_p$ = pooled standard deviation of dry matter loss rate (% d$^{-1}$)
$\Sigma m_{CO_2,V}$ = accumulated mass of respired $CO_2$ inside a RC (mg $CO_2$ m$^{-3}$)
$\Sigma m_{CO_2,s}$ = accumulated specific mass of respired $CO_2$ (mg $CO_2$ kg$^{-1}$ dry matter)
$\phi$ = relative humidity (% RH)
$\phi_e$ = equilibrium relative humidity (% RH)