

# Effect of Field Treatment on Microfloral Respiration and Storability of Canola under Different Storage Conditions

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**How to cite this paper:** Jian, F.J., Tang, P.A., Al Mamun, Md.A. and Jayas, D.S. (2019) Effect of Field Treatment on Microfloral Respiration and Storability of Canola under Different Storage Conditions. *American Journal of Plant Sciences*, 10, 1989-2001. <https://doi.org/10.4236/ajps.2019.1011139>

**Received:** October 9, 2019

**Accepted:** November 12, 2019

**Published:** November 15, 2019

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## Abstract

Whether different field treatments such as straight cut, swathing, or pre-harvest aid application can influence the canola storage is the critical information for growers. The effect of these different field treatments on the infection and development of microflora on canola seeds with 9%, 11%, and 14% moisture content at 20°C, 25°C, 30°C and 35°C was determined. To evaluate the microfloral infection and development, concentration of CO<sub>2</sub> and O<sub>2</sub> in 150 g canola bulks were measured every 3 d. At the beginning and end of the study (66 d), the seed moisture content, germination, visible mold, invisible fungal infection, and yellow seed count were measured. The swathed and nature ripened canola had a slightly higher respiration rate at ≥30°C and ≥ 2 wk than the seeds with other field treatments. The swathed canola had a marginally lower initial germination and higher germination at 66 d. The Glyphosate treated and nature ripened canola seeds had a slightly higher chance of visible model development. However, all these differences were not significant at  $\alpha = 0.05$  level and different field treatments and storage conditions did not influence the yellow seed count.

## Keywords

Canola, Pre-Harvest Treatment, Pre-Harvest Aid, Respiration, Safe Storage

## 1. Introduction

Canada annually produces about 20 million tonnes of canola, which contributes \$26.7 billion dollars (CAD) to Canadian economy and is about one quarter of all farm income. Farmers harvest canola seeds by swathing, straight cutting, or straight cutting with the application of pre-harvest aids (referred to as field

treatment) such as Glyphosate (N-(phosphonomethyl)glycine), Heat LQ (Group 14 saflufenacil), and Reglone (Diquat ion). These harvested seeds are stored in farm bins for up to one year. Under unsafe storage conditions, the stored seeds might be infected by microflora, which results in high respiration rates and quality reduction.

Glyphosate, Heat LQ, and Reglone are registered as pre-harvest perennial weedicide, desiccants and annual weed control, and fast dry down desiccants, respectively in Canada [1]. All of these registered chemicals can be used as pre-harvest aids because they can kill and/or desiccate crops and weeds. Glyphosate is absorbed by leaves and stems, transported within the plant, and prevents the production of a plant-specific enzyme (5-enolpyruvylshikimate-3-phosphate synthase) which leads to plant death by starvation [2]. This slow process requires 1 - 3 wk and it may end up in the seed as herbicide residues if Glyphosate is applied earlier when the plant is still actively translocating nutrients to the seed kernels. Reglone does not hasten crop maturity, but ruptures the outer layer of cellular membrane of plant cells. Reglone has an acting period of 4 - 7 d and the plant dries down faster than it would without Reglone application when the plant is exposed to sun. After Heat LQ is rapidly absorbed by root, leaves, and stems, it inhibits protoporphyrinogen oxidase, which results in cell membrane damage and leads to plant death and drydown. Heat LQ has an acting period of 3 - 5 d and provides a broad spectrum weed control and improves crop uniformity for harvesting. It has been reported that germination of canola is not influenced by Reglone and Heat LQ [3], but Glyphosate reduces germination if it is applied when the pods are green and when seed moisture content is high [1]. Therefore, different harvest methods and use of chemical desiccants may influence the physiological status of the plants and seeds, hence affect the initial storage condition of canola.

The initial storage condition includes oilseed temperature, moisture content (MC) (in this manuscript MC are on wet basis unless noted otherwise), relative humidity (RH), maturity, pre-harvest treatment, seed vigor, and microfloral infection. After canola is harvested and binned, canola may have a high respiration rate for up to 6 wk [4] [5] [6]. A high percentage of green seeds might accelerate the respiration and deterioration of stored canola [7] [8] [9]. Higher respiration of stored canola will produce heat and water which might result in hotspot development [4]. Jian *et al.* (unpublished data) found canola seeds with different field treatments had different equilibrium RHs at the same moisture content and temperature of the seeds. The swathed canola had a significant lower initial germination at all storage conditions studied and higher germination at the storage condition of 35°C and 75% RH than any canola seeds with different field treatments at 12 wk. At 93% RH, the yellow seed count increased with the storage time except for the swathed canola. Different field treatments had different fungal species with different initial infections.

Respiration of seeds under different stored conditions is usually determined by measuring CO<sub>2</sub> production and O<sub>2</sub> consumption [5] [10]. These determined

CO<sub>2</sub> and O<sub>2</sub> concentrations indicate the development of microflora infecting the stored seeds because grain has negligible respiration rate under safe storage conditions [11] and most, if not all, the measured CO<sub>2</sub> is produced by the microflora [5] [12]. Respiration rates of microflora in stored grain are usually much higher than that of the dry grain except when wet seeds sprout [13]. Therefore, CO<sub>2</sub> concentrations measured in the interstitial air can facilitate early detection and level of spoilage in storage grain bulks [14]. It is not known whether different field treatments such as straight cutting, swath, or desiccant application can influence the respiration of the seeds under different storage conditions.

The aim of this study was to measure the respiration of 9%, 11%, and 14% MC canola seeds with different field treatments and stored at 20°C, 25°C, 30°C, and 35°C. The measured respirations were used to answer the question: whether the canola seeds with different field treatments have different spoilages or not? The field treated canola seeds were: the canola plants swathed (referred to as SW), Glyphosate applied + straight cut (referred to as GL), Heat LQ and Glyphosate applied + straight cut (referred to as HG), Reglone applied + straight cut (referred to as RE), and natural ripening + straight cut (referred to as NR). To evaluate the storability of the seeds under different storage conditions, MC, germination, visible and invisible mold, and yellow seed count were determined.

## 2. Materials and Methods

### 2.1. Field Treatment and Seed Preparation

Canola cultivar Bayer L233P was seeded on an 80-acre field located at about 20 km north of Winnipeg. Standard field practices including seeding date, pest and weed management, and fertility were applied to the field to maximize the canola production. The canola plants at different locations of the field had a similar growth rate and height before the field treatment. For the convenience of the pre-harvest treatment, the field was evenly divided into three zones in the North-East direction, and each zone was further divided into 10 plots in the same direction [15]. The width of a plot was two passes of a chemical sprayer or combine (10.7 to 16.7 m). To avoid any treatment overlap, the canola plants between two passes (3 m) were cut before the chemical application. The following five pre-harvest treatments were applied to each zone (two plots per treatment) in the order of North to South direction: GL, RE, SW, HG, and NR. Therefore, there were total three replicates (including 6 passes) for each pre-harvest treatment. The application rates of the Reglone, Heat and Glyphosate, and Glyphosate were 25, 15, and 10 gals/acre (233.85, 140.31, and 93.54 L/ha), respectively. The application times of Reglone, Heat and Glyphosate, and Glyphosate were 5, 9, and 15 d before harvest, respectively. The wind speed during the chemical application period was less than 38 km/h. These application times and rates were recommended by the chemical manufacturer (Bayer CropScience Inc., Morrisville, NC, USA). The canola was swathed on August 28, 2017. The harvest times of the other four pre-harvest treatments were on August 29 and 30, 2017. Harv-

est time was decided based on the farmer's experience, recommendations from the Canola Council of Canada, and from the pre-harvest aid manufacturer (Bayer CropScience Inc., Morrisville, NC, USA). A 35' MacDon draper header (MacDon, Winnipeg, MB, Canada) was used to straight cut the canola plants. Swathed treatments were harvested with a John Deere Pick-up header (John Deere S690, Grand Detour, IL, USA). All plots were combined with a John Deere S670 (Grand Detour, IL, USA). The auto-steer and GPS were used to ensure accuracy of spraying and harvesting. To limit the influence of field operation on the storage study, about 100 kg canola seeds were collected from each pass for each treatment, and the collected canola seeds with the same pre-harvest treatment and from the three zones (about 600 kg for each pre-harvest treatment) were mixed before lab study. All the harvested canola from different pre-harvest treatments had  $10.5\% \pm 0.5\%$  moisture content (MC).

After the harvested seeds were delivered to the lab, the 600 kg seeds for each pre-harvest treatment were cleaned using a hand sieve (No. 14, 5.56 mm openings) to separate larger materials (such as chaff) from the seeds, and mixed again by using a lab-fabricated mixture. After mixing, the canola MC was measured using ASABE standard [16] and the MC of the canola was  $10.5\% \pm 0.1\%$ . To achieve the desired MC of the canola, 150 kg of the seeds from each pre-harvest treatment were spread on a floor in a lab room. The lab room was at  $27^\circ\text{C} \pm 2^\circ\text{C}$  and  $40\% \pm 5\%$  relative humidity (RH). The canola thickness on the floor was about 1 cm and a rake was used to mix the canola every 2 d. The canola MC was measured every 1 d and 75 kg canola seeds were bagged in double layer plastic bags when the MC was  $9.0\% \pm 0.1\%$ . To achieve 11.0% MC canola, 1.264 L distilled water was added to 225 kg canola with  $10.5\% \pm 0.1\%$  MC and mixed in the mixture for 0.5 h [17]. One week later, this conditioned canola was mixed again for 0.5 h. The same method was used to produce the  $14.0\% \pm 0.1\%$  MC canola. All the conditioned and bagged seeds were stored at  $5^\circ\text{C} \pm 1^\circ\text{C}$  for at least 2 wk before using. The MC of these conditioned canola seeds was measured again before the test and the MCs of these canola seeds were  $9.0\% \pm 0.1\%$ ,  $11.0\% \pm 0.1\%$ , and  $14.0\% \pm 0.1\%$  (standard error was reported when a mean value was presented in this article). These adjusted MCs were reported as the initial MCs in this article.

## 2.2. Measurement of CO<sub>2</sub> and O<sub>2</sub> Concentration

Methods reported by Jian *et al.* [5] were used to measure the interstitial CO<sub>2</sub> and O<sub>2</sub> concentration of the canola seeds. The same Erlenmeyer flasks (300 mL) and setup as used by Jian *et al.* [5] were used in this study and 150 g canola seeds with a desired moisture content were filled in a flask. The flasks were sealed with rubber stoppers and kept in environmental chambers (Conviron CMP3244, Controlled Environments Ltd., Winnipeg, MB, Canada) and temperatures of the environmental chambers were set at  $20^\circ\text{C} \pm 1^\circ\text{C}$ ,  $25^\circ\text{C} \pm 1^\circ\text{C}$ ,  $30^\circ\text{C} \pm 1^\circ\text{C}$ , and  $35^\circ\text{C} \pm 1^\circ\text{C}$ . Concentrations of CO<sub>2</sub> and O<sub>2</sub> of gas samples were determined us-

ing a procedure described by Jian *et al.* [5] and a Perkin Elmer Gas chromatograph (Model: Clarus 420. ON, Canada) equipped with a thermal conductivity detector was used. The setting of the chromatograph was: 30 mL/min helium carrier gas, 150°C detector temperature, 70°C oven temperature, a 6-ft (1.85-m) column (2 mm inside-diameter) packed with Hayesep N 60/80 mesh for CO<sub>2</sub> measurement, and a 9-ft (2.78-m) 13 × molecular sieve 40/60 mesh column for O<sub>2</sub> measurement. The gas chromatograph was calibrated using high purity mixtures of CO<sub>2</sub> in N<sub>2</sub> or O<sub>2</sub> in helium (Matheson Tri-Gas, Morrow, Georgia, USA). Based on the CO<sub>2</sub> concentrations and their corresponding areas calculated by the integrator of the Perkin Elmer gas chromatograph, a regression equation was developed, and this regression equation was used to determine the CO<sub>2</sub> concentration inside each flask. The same method was used to determine the O<sub>2</sub> concentration.

Concentrations of CO<sub>2</sub> and O<sub>2</sub> in each flask were measured every 3 d by sampling 4 mL gases (2 mL gas sample for CO<sub>2</sub> and 2 mL gas sample for O<sub>2</sub> measurement). To manage the work load, the respiration of seeds with 9.0% ± 0.1%, 11.0% ± 0.1%, and 14.0% ± 0.1% MCs and stored at 25°C ± 1°C, and the seeds with 11.0% ± 0.1% MC and stored at 20°C ± 1°C, 30°C ± 1°C, and 35°C ± 1°C were measured. After the gas was sampled, canola seeds inside the flask were slowly transferred to another flask and then transferred back to the same flask by using a funnel (6.0-cm high and 6.0-cm diameter top opening and 0.8-cm outer-diameter spout) [5]. This transfer procedure would replace the CO<sub>2</sub> and O<sub>2</sub> inside the flasks with room air. The respiration measurement was stopped at 66 d of the storage time.

### 2.3. Measurement of Storage Parameters

Before and at the end of this study, the following parameters were measured: MC [16], seed germination (percentage), visible mold (fungal species and infection percentage), invisible fungal infection (fungal species and infection percentage), and green/yellow seed count (percentage). About 10 g seeds were used to determine the MC.

Germination was determined by putting 25 kernels in a 9 cm diameter petri dish with a filter paper and 5 mL distilled water [18]. The kernels were incubated at room temperature (25°C ± 2°C), and the number of sprouted kernels was counted 7 d later. The germination was calculated using the numbers of the sprouted kernels divided the 25 seeds and multiplied by 100.

To determine the visible mold infection, 25 kernels (without incubation) were observed under the dissecting microscope. The mold species were identified based on the color and shape of the mold colony. A kernel was count as an infected kernel if one or more than one colonies were found on the kernel, and the percentage of visible infection was calculated using the numbers of infected seeds divided the 25 seeds and multiplied by 100. To determine the invisible mold, 25 kernels were put in a 9 cm diameter petri dish with a filter paper and 5

mL 7.5% aqueous sodium chloride solution. After 7 d of incubation at the room temperature, invisible mold was identified using a dissecting microscope based on the color and shape of the microfloral colony [19] [20]. The percentage of the microfloral infection of each fungal species was calculated using the numbers of infected seeds divided the 25 seeds and multiplied by 100.

Green and yellow seed counts were determined by following the Official Grain Grading Guide of Canadian Grain Commission [21]. Canola seeds were crushed using a plastic paddle (2 in diameter ×10 inch length) and then masking tapes were used to keep 200 seeds on the paddle. A vinyl roller was used to crush the seeds, and the number of seeds (on the tapes) with green and yellow colour was counted and percentage of the yellow seeds was calculated and reported as the yellow seed count.

## 2.4. Data Analysis

A completely randomized design with three replicates at each treatment was conducted. To check whether the seeds with different field treatments had different respiration at the same storage condition and time, Paired t-test was conducted to compare the mean CO<sub>2</sub> concentration of the seeds with different field treatments but at the same storage condition [22]. Tukey tests were conducted to compare the CO<sub>2</sub> concentration associated with different field treatments but stored at the same condition and time. Student t-test was conducted to compare a storage parameter of the seeds at the same field treatment and same storage condition between at 0 and 66 d of storage time.

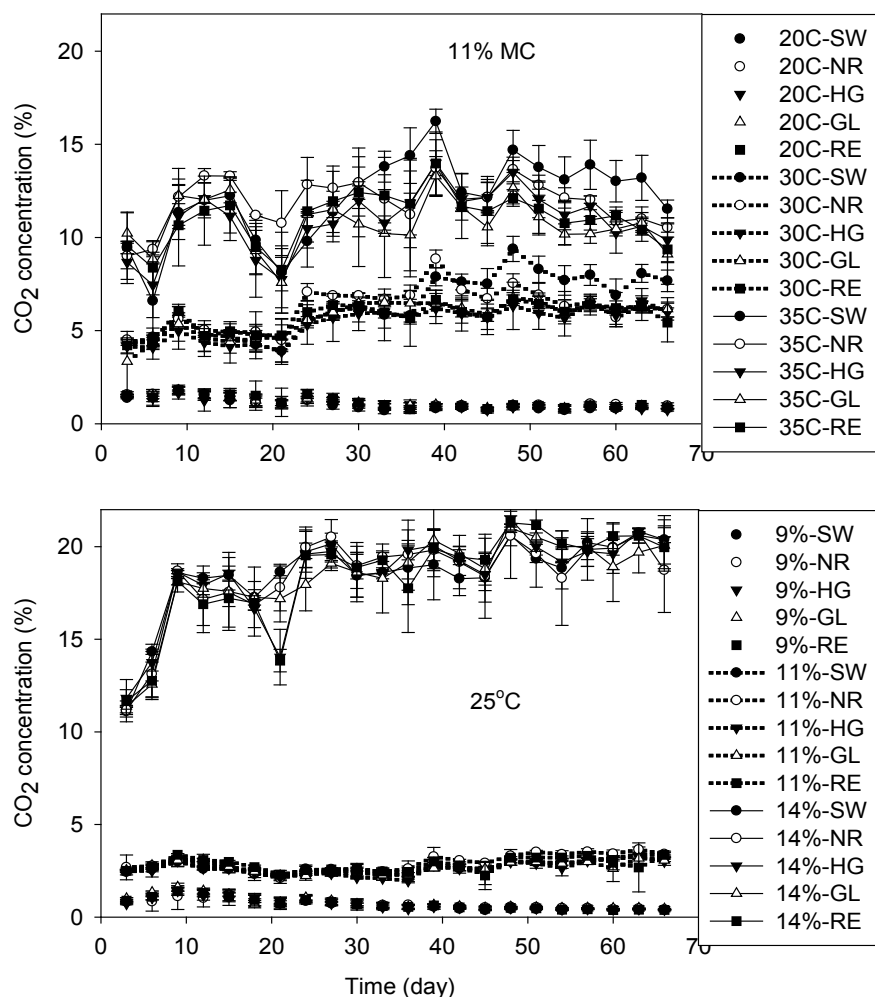
## 3. Results and Discussion

### 3.1. Moisture Content

The moisture content of the seeds at 66 d was not significantly different from their initial moisture contents (Student t-test, all  $t \leq 1.902$ , all  $p \geq 0.130$ ,  $df = 4$ ) and there was no significant difference among the seeds with different field treatments and stored at the same condition (Tukey test, all  $F \leq 3.158$ ,  $p \geq 0.064$ ). However, the standard error of the moisture content of the seeds was increased from the initial  $\leq 0.1\%$  to the final 0.3% to 0.4%. Therefore, the canola seeds with different field treatments were approximately stored at the constant moisture contents in this study.

### 3.2. Concentration of CO<sub>2</sub> and O<sub>2</sub>

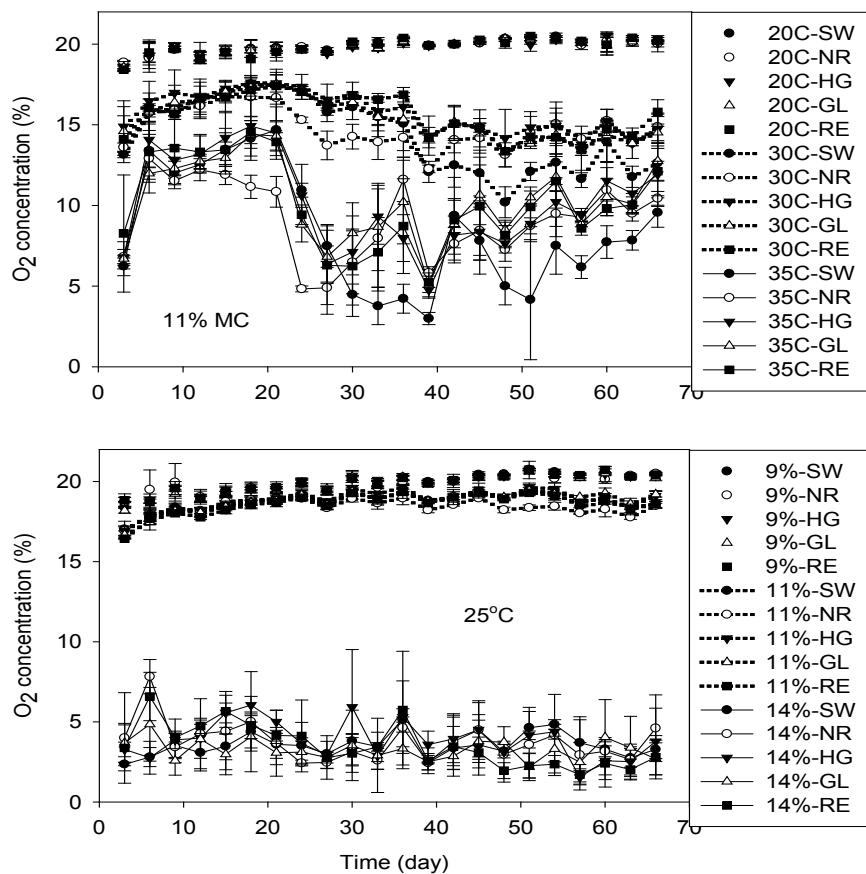
The CO<sub>2</sub> concentrations in the interstitial air of the seeds stored at higher temperatures and/or higher MCs were higher than that at lower temperatures and lower MCs (Figure 1). This was consistent with the literatures [5] [6] [14]. The O<sub>2</sub> concentration was low when the CO<sub>2</sub> concentration was high (Figure 2), and the sum of the CO<sub>2</sub> and O<sub>2</sub> concentrations was about 21% [5]. This indicated the microflora consumed one O<sub>2</sub> and produced one CO<sub>2</sub> at the same time, and carbohydrate was mostly used as the substrate and consumed by microflora during



**Figure 1.** Measured CO<sub>2</sub> concentration of canola seeds with 11% moisture content and stored at different temperatures (top) or the seeds with 9, 11, and 14% moisture contents at 25°C (bottom). The plants were swathed (SW), natural ripening + straight cut (NR), Heat LQ and Glyphosate applied + straight cut (HG), Glyphosate applied + straight cut (GL), and Reglone applied + straight cut (RE).

their respiration [23]. The main composition of rapeseed (canola) hull is non-lignified biomass which contains about 60% holocellulose, 14% protein, and minimum amount of lipids [24]. These main carbohydrate components might be used by the microflora in the tested period, and the microflora did not consume or consumed a negligible amount of lipids which are located inside the hull of the canola seeds.

There were no significant differences of the CO<sub>2</sub> concentrations produced in the 14% MC seeds with different field treatments (Table 1), while there were significant differences at other storage conditions in 52% cases. These significant differences were caused by the fluctuated CO<sub>2</sub> concentrations (Figure 1). For example, the mean CO<sub>2</sub> concentrations of the SW, NR, HG, GL, and RE canola with 11% MC and stored at 35 and at 39 d were  $16.2\% \pm 0.7\%$ ,  $13.7\% \pm 1.5\%$ ,  $14.0\% \pm 0.7\%$ ,  $13.3\% \pm 1.0\%$ , and  $14.0\% \pm 1.7\%$ , respectively; and at 42 d were



**Figure 2.** Measured O<sub>2</sub> concentration of canola seeds with 11% moisture content and stored at different temperatures (top) or the seeds with 9%, 11%, and 14% moisture contents at 25°C (bottom). The plants were swathed (SW), natural ripening + straight cut (NR), Heat LQ and Glyphosate applied + straight cut (HG), Glyphosate applied + straight cut (GL), and Reglone applied + straight cut (RE).

**Table 1.** The p values of the Paired t-test to compare the CO<sub>2</sub> concentration produced by canola with different field treatments and under the same storage conditions.

Field treatment	Storage conditions					
	11% MC 20°C <sup>‡</sup>	11% MC 25°C <sup>‡</sup>	11% MC 30°C <sup>‡</sup>	11% MC 35°C <sup>‡</sup>	9% MC 25°C <sup>‡</sup>	14% MC 25°C <sup>‡</sup>
SW-NR <sup>†</sup>	<0.001**	<0.001**	0.395	0.616	0.523	0.533
SW-HG <sup>†</sup>	0.047*	0.016*	<0.001**	<0.001**	0.089	0.773
SW-GL <sup>†</sup>	<0.001**	0.784	0.002*	0.002*	0.001**	0.366
SW-RE <sup>†</sup>	<0.001**	0.046	0.008*	0.002*	0.004*	0.611
NR-HG <sup>†</sup>	0.034*	<0.001**	0.001*	<0.001**	0.749	0.910
NR-GL <sup>†</sup>	0.366	<0.001**	<0.001**	<0.001**	0.061	0.645
NR-RE <sup>†</sup>	0.450	0.106	0.002*	<0.001**	0.510	0.828
HG-GL <sup>†</sup>	0.028*	0.138	0.002*	0.468	0.029*	0.580
HG-RE <sup>†</sup>	0.009*	0.001*	<0.001**	0.485	0.644	0.634
GL-RE <sup>†</sup>	0.107	0.008*	0.580	0.155	0.007*	0.875

<sup>†</sup>Comparison between field treatments (Paired t-test). SW, NR, HG, GL, and RE are the swathed, natural ripening + straight cut, Heat and Glyphosate applied + straight cut, Glyphosate applied + straight cut, and Reglone applied + straight cut canola. <sup>‡</sup>The p value of the Paired t-test is presented in the columns at each of the storage conditions. \*significant at  $\alpha = 0.05$  level.



12.4%  $\pm$  1.0%, 12.0%  $\pm$  0.3%, 12.0%  $\pm$  0.7%, 11.6%  $\pm$  0.7%, and 11.7%  $\pm$  1.7%, respectively. These fluctuations might not occur inside grain storage bins because the interstitial air inside the seeds were replaced for every 3 d during this study and high CO<sub>2</sub> and low O<sub>2</sub> concentrations will inhibit the aerobic respiration in bins if the grain bulks are not aerated.

There was no significant difference of CO<sub>2</sub> concentration associated with the seeds with different field treatments and stored at the same condition and time (Tukey test, all  $F \leq 0.525$ ,  $p \geq 0.720$ ,  $df = 14$ ). Therefore, the seeds with different field treatments had the similar respiration rate. At 35°C, the NR canola with 11% MC at 14 to 27 d and the SW canola with 11% MC at 33 to 66 d had a consistent higher CO<sub>2</sub> concentration than that of the canola seeds treated with other field treatments (Figure 1). These also occurred for the NR and SW canola at 30°C. The high concentration of CO<sub>2</sub> indicated a faster growth of microflora. Therefore, the swathed and nature ripened canola had a slightly higher respiration rate at higher temperatures (higher than 30°C) when storage time is longer than 2 wks. This might be caused by the storage microflora infection before the swathed or nature ripened canola was collected from the field.

### 3.3. Germination

The initial germinations of the seeds were  $\geq 96.0\% \pm 3.9\%$  except the swathed canola seeds (Table 2). One of the replicates of the SW canola seeds had 84% germination. This low initial germination might be caused by the microflora infection on the field before the swathed canola was collected. The seeds stored at 25°C had  $\geq 92\%$  germination (Table 2). The germinations of the 11% MC seeds with different field treatments and stored at 35°C were significantly decreased at 66 d from the initial germination (Student t-test, all  $t \geq 6.038$ , all  $p \leq 0.004$ ,  $df = 4$ ). The decrease of the germination did not influence the production of CO<sub>2</sub> and O<sub>2</sub> consumption [25]. These results were consistent with the literatures [6] [20]. The SW canola at the end of this study had a slightly higher germination than the seeds with the other field treatments (Table 2), but this high germination

**Table 2.** Germination of the seeds with different field treatments and at different storage conditions.

Storage condition <sup>†</sup>		Field treatments <sup>‡</sup>				
T and time <sup>†</sup>	MC (%)	SW	NR	HG	GL	RE
Initial (0 day storage time)	9	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	98.7 $\pm$ 2.3	100.0 $\pm$ 0.0	98.7 $\pm$ 2.3
	11	97.3 $\pm$ 4.6	98.7 $\pm$ 2.3	100.0 $\pm$ 0.0	96.0 $\pm$ 3.9	97.3 $\pm$ 2.3
	14	94.7 $\pm$ 9.2	97.3 $\pm$ 2.3	97.3 $\pm$ 2.3	97.3 $\pm$ 4.6	98.7 $\pm$ 2.3
25°C at 66 d	14	98.0 $\pm$ 2.8	96.0 $\pm$ 4.0	92.0 $\pm$ 6.9	98.7 $\pm$ 2.3	96.0 $\pm$ 6.9
35°C at 66 d	11	50.7 $\pm$ 4.6	45.3 $\pm$ 6.1	36.0 $\pm$ 6.9	48.0 $\pm$ 10.6	45.3 $\pm$ 8.3

<sup>†</sup>T = temperature, Time = storage time, MC = moisture content. The germination of seeds at other storage conditions (20°C at any moisture content, 25°C at 11, and 9% moisture content, and 30°C at 11% moisture content) with 66 d of storage time were higher than 94.7%. <sup>‡</sup>SW, NR, HG, GL, and RE are the swathed, natural ripening + straight cut, Heat and Glyphosate applied + straight cut, Glyphosate applied + straight cut, and Reglone applied + straight cut canola.

of SW seeds was not significantly different from the seeds with the other field treatments (Tukey test,  $F = 0.531$ ,  $p = 0.716$ ,  $df = 14$ ).

### 3.4. Visible and Invisible Mold

No visible mold was found at the beginning of the storage. At 66 d, *Aspergillus candidus* was the only visible mold infecting the kernels, and no visible mold was found on the kernels stored at 20°C, or 9 and 11% MC seeds at 25°C. These results were consistent with the literatures [20]. The maximum infection percentage of visible mold was 12% which was observed on NR canola with 11% MC and at 35°C. At 35°C, all the GL canola samples had visible mold. There was no significant difference of the infection percentage among the seeds with different field treatments. Therefore, field treatment might not influence the visible mold development, and GL and NR canola seeds had a slightly higher chance of visible mold development.

The initial infection of the invisible mold was *Alternaria alternata*. The dominant fungal species at 66 d was *Aspergillus glaucus*. The other occasionally infected species were *Penicillium spp.* and *A. candidus*. The species of microflora were consistent with that reported by Jian *et al.* [5] and Sun *et al.* [20]. Almost 100% of kernels with any field treatment were infected by invisible mold at 66 d of the storage time (Table 3). These results were consistent with the literatures [5] [6] [20]. Therefore, different field treatments might not influence the microflora infection.

There were no green seeds in all of the samples. The initial yellow seed count of seeds with different field treatments was not significantly different. The initial yellow seed count of the seeds with different field treatments was  $0.0044\% \pm 0.0014\%$ . The yellow seed count was not significantly different in the seeds with different field treatments and stored at the same storage condition at 66 d

**Table 3.** Percentage of infection (%) of the visible mold (*Aspergillus candidus*) and invisible mold on canola seeds with different field treatments and at different storage conditions with 66 d of storage time.

Mold	Storage condition <sup>†</sup>		Field treatments <sup>‡</sup>				
	T (°C)	MC (%)	SW	NR	HG	GL	RE
Visible <sup>§</sup>	25	14	0.0 ± 0.0	1.3 ± 1.3	1.3 ± 1.3	0.0 ± 0.0	1.3 ± 1.3
	30	11	1.3 ± 1.3	0.0 ± 0.0	0.0 ± 0.0	2.7 ± 2.7	0.0 ± 0.0
	35	11	1.3 ± 1.3	4.0 ± 4.0	0.0 ± 0.0	4.0 ± 0.0	2.7 ± 2.7
Invisible <sup>  </sup>	25	9	100 ± 0.0	100 ± 0.0	90.7 ± 3.5	96.0 ± 2.3	98.7 ± 1.3
	20	11	89.3 ± 1.3	89.3 ± 1.3	97.3 ± 1.3	96.0 ± 2.3	94.7 ± 1.3

<sup>†</sup>T = temperature, and MC = moisture content. <sup>‡</sup>SW, NR, HG, GL, and RE are the swathed, natural ripening + straight cut, Heat and Glyphosate applied + straight cut, Glyphosate applied + straight cut, and Reglone applied + straight cut canola. <sup>§</sup>The percentage of visible mold infection at other storage conditions (20°C at any moisture content, and 25°C at 11 and 9% moisture content) with 66 d of storage time were 0.0% ± 0.0%. <sup>||</sup>The percentage of invisible mold infection at other storage conditions (20°C at any moisture content, and 25°C at 11% and 9% moisture content) with 66 d of storage time were 100.0% ± 0.0%.

(Tukey test, all  $F \leq 2.514$ , and all  $p \geq 0.101$ ,  $df = 17$ ). The yellow seed count was not significantly different in the seeds with different storage conditions at 66 d and the same field treatment (Tukey test, all  $F \leq 2.871$ , and all  $p \geq 0.062$ ,  $df = 17$ ). There was no significant difference of the yellow seed count between at the 0 and 66 d for each field treatment and each storage condition (Student t-test, all  $t \leq 1.512$ , all  $p \geq 0.205$ ,  $df = 4$ ). Green seed count of canola at the harvest is not influenced by preharvest applications of glyphosate [1]. Therefore, different field treatments and storage conditions did not influence the yellow seed count. To the best of our knowledge, there is no study on the yellow seed reduction during storage periods. Several studies were conducted on green seed counts and the chlorophyll content. Canada swathed and then dried at 40°C can reduce chlorophyll content [26]. Air ventilation of stored canola can decrease the chlorophyll content in seeds [27]. Our study on the yellow seed count was inconsistent with this conclusion.

#### 4. Conclusions

1) Even though the seeds with different field treatments had not a significant difference in respiration rate, the swathed and nature ripened canola had slightly higher respiration rate at higher temperatures (higher than 30°C) when storage time was longer than 2 wks.

2) The swathed canola had a slightly lower germination at the beginning and higher germination at 66 d than the seeds with the other field treatments, but this high germination was not significant.

3) Field treatments did not influence the visible and invisible mold development, but Glyphosate treated and nature ripened canola seeds had a marginally higher chance of visible mold development.

4) Different field treatments and storage conditions did not influence the yellow seed count.

#### Acknowledgements

We thank the Prairie Agricultural Machinery Institute (PAMI), Natural Sciences and Engineering Research Council of Canada (NSERC) for partial funding of this study, and Canada Foundation for Innovation, Manitoba Research Innovation Fund, and several other partners for creating research infrastructure. The authors are grateful to Colin Demianyk for preparing the saturated salt solutions.

#### Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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