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# Effect of pre-harvest treatments on equilibrium moisture contents and safe storage of canola

Fuji Jian\*, Md Abdullah Al Mamun and Digvir S. Jayas

*Department of Biosystems Engineering, University of Manitoba, Winnipeg, MB, R3T 5V6, Canada.*

*Corresponding Author: [Fuji.Jian@umanitoba.ca](mailto:Fuji.Jian@umanitoba.ca)*

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## ABSTRACT

For safe storage of canola seeds, the effect of different pre-harvest treatments on seed storability should be determined. Safe storage times of canola seeds (Bayer L233P) with the following five pre-harvest treatments were evaluated: swathed, Glyphosate application + straight cut, Heat and Glyphosate application + straight cut, Reglone application + straight cut, and natural ripening + straight cut. The pre-harvest treated seeds were stored at 20, 25, 30, or 35°C and 52, 63, 75, or 93% relative humidity (RH). The following parameters were measured to estimate the safe storage time: seed equilibrium moisture content (EMC), germination, fatty acid value (FAV), yellow seed count, and invisible mould. The measured EMCs were compared with the EMCs predicted by the equations recommended by the ASABE standard. Different pre-harvest treatments resulted in different desorption properties of canola. None of the ASABE equations was able to predict the measured EMCs correctly. Different pre-harvest treatments had different initial fungal infections. However, these differences did not affect the fungal infection, FAV, germination, and yellow seed counts, with some exceptions for swathed canola in the storage period. The yellow seed count decreased under safe storage conditions (lower than 75% RH and 25°C), but increased at 93% RH and 25°C except for the swathed canola. Therefore, canola seeds with different pre-harvest treatments had a similar storability at below 75% RH or below 30°C. However, the spoilage rates of canola with different pre-harvest treatments at storage conditions of high temperatures ( $\geq 30^\circ\text{C}$ ) and high RHs ( $\geq 75\%$ ) were different.

## KEYWORDS

Canola, pre-harvest aid, pre-harvest treatment, safe storage time, safe storage moisture content, yellow seed count.

## RÉSUMÉ

Pour un entreposage sécuritaire des graines de canola, l'effet de différents traitements en pré-récolte sur leur conservation doit être déterminé. Des temps d'entreposage sécuritaires ont été évalués pour des graines de canola (Bayer L233P) ayant subies les cinq traitements pré-récolte suivants : andainage, pulvérisation au glyphosate + coupe directe, pulvérisation au Heat et au glyphosate + coupe directe, pulvérisation au Reglone + coupe directe et murissement naturel + coupe directe. Les graines traitées ont été entreposées à 20, 25, 30 ou 35 °C et à 52, 63, 75 ou 93 % d'humidité relative (HR). Les paramètres suivants ont été mesurés pour estimer le temps d'entreposage sécuritaire : teneur en eau d'équilibre des graines [EMC], la germination, la valeur en acides gras [FAV], le nombre de graines jaunes et la moisissure invisible. Les valeurs d'EMC mesurées ont été comparées à celles prédites par les équations recommandées par les standards de l'ASABE. Les différents traitements en pré-récolte ont entraîné différentes propriétés de désorption du canola. Aucune des équations de l'ASABE n'a été capable de prédire correctement les valeurs d'EMC mesurées. Les différents traitements pré-récolte avaient des contaminations fongiques initiales différentes. Toutefois, ces différences n'ont pas eu d'effet sur la contamination fongique, la FAV, la germination et le décompte de graines jaunes, avec quelques exceptions durant la période d'entreposage du canola coupé en andains. Le nombre des graines jaunes a diminué durant des conditions sécuritaires d'entreposage inférieures à 75 % HR et 25 °C, mais a augmenté à 93 % HR et 25 °C, à l'exception du canola andainé. Ainsi, le canola ayant subi différents traitements pré-récolte s'est conservé de manière semblable lorsque les conditions d'entreposage étaient inférieures à 75 % HR ou 30 °C. Toutefois, les taux de perte de graines de canola ayant subi différents traitements pré-récolte étaient différents à des conditions d'entreposage de températures et d'HR élevées ( $\geq 30^\circ\text{C}$  et  $\geq 75\%$ ).

## MOTS CLÉS

Canola, aide à la pré-récolte, traitement pré-récolte, période sécuritaire d'entreposage, humidité relative sécuritaire pour l'entreposage, nombre de graines jaunes.

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## CITATION

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## INTRODUCTION

Canadian farmers plant about 9.5 million hectares of canola and produce about 19 million metric tonnes of canola seeds every year. Canola seeds are traditionally swathed, binned, and stored for up to one year. The advantages of swathing are that it can hasten the drying rate in the field, even out ripening, improve seed maturity, and reduce the risk of losses from wind and hail (Cenkowski et al. 1989a). Disadvantages of swathing include a need for specialized equipment and additional time required to pick up the swathed plants. Also, the swathed canola plants may be rewetted by rain (Canola Council of Canada 2019) and also swathing at the wrong time may lower yield and reduce quality, i.e., the appearance of green stalks, regrowth, and uneven ripening (Cenkowski et al. 1993). Applications of desiccants or pre-harvest weedicides can help with timely defoliation, even crop ripening, and can "reduce yield losses caused by harvest delays as well as dockage and green seeds or weeds" (Larson et al. 2008). Therefore, straight cutting (direct combining) with the spraying of pre-harvest aids such as Glyphosate (N-(phosphonomethyl) glycine), Heat (Heat® LQ, Group 14 Saflufenacil), or Reglone (Diquat ion) has been gradually accepted by farmers (Simundsson et al. 2017). Even though shatter losses associated with the straight cut may be higher than swathing, the straight cutting with the application of pre-harvest aids lowers the cost as there is no need to purchase the swathing equipment and shortens the harvest time, which becomes necessary in northern regions of the world such as Canada where the growing season is short.

Glyphosate is registered for pre-harvest perennial weed control in Canada and is used as a herbicide and crop desiccant. Since its discovery in the early 1970's it "has become the world's most widely used herbicide because it is efficacious, economical and environmentally benign" (Powles 2008). Glyphosate is absorbed by leaves and stems, transported within the plant, preventing the production of a plant-specific enzyme (5-enolpyruvylshikimate-3-phosphate synthase). This leads to plant death by starvation (Jordan and Donaldson 1996). This herbicide is used when the seed moisture is less than 30%, and the transport of nutrients from the leaves, stems, and spike to the kernel is complete. Glyphosate can end up in seed kernels as herbicide residue if it is applied to plants, which are still actively translocating nutrients to kernels. The residue can affect germination and seedling vigour. "Canola is very efficient at moving Glyphosate or other systemic compounds into the seed before physiological maturity. Excessive pesticide residues in the seed can result in export problems" (Canola Council of Canada, 2019). Reglone is a contact desiccant that works by rupturing the outer layer of the cellular membrane of plant cells and allowing the plant to dry down naturally faster than it would without Reglone application when plants are exposed to sunlight (Syngenta Canada Inc. 2016). Reglone stops plants from maturing. Therefore, plants can have higher green seed levels if the plants are applied prematurely (Canola Council of Canada,

2019). Heat (Heat® LQ) is a water-based suspension of concentrated herbicide (Saflufenacil) for broadleaf weed control (BASF Canada Inc. 2014). After it is rapidly absorbed by root, leaves, and stems, it inhibits protoporphyrinogen oxidase, which results in cell membrane damage and leads to plant death. Heat is recommended for pre-seed, pre-emergent, and pre-harvest applications (BASF Canada Inc. 2014).

Different harvest methods and use of desiccants may influence the initial storage condition of canola, which is one of the most important factors influencing the seed deterioration of stored oilseeds (Jian and Jayas 2014; Rukunudin et al. 2004). The initial storage condition includes oilseed temperature, moisture content, maturity, and pre-harvest treatment. After canola seeds are harvested and binned, they may have a high respiration rate for up to 6 weeks (Jian et al. 2014a; Jian et al. 2014b; Pronyk et al. 2004). This high respiration rate might result in a phenomenon referred to as "sweating." A high percentage of green seeds might accelerate the sweating process (Cenkowski et al. 1989b; Johnson-Flanagan et al. 1994). Sweating might result in the development of hotspots resulting from an increase in temperature and moisture content of seeds (Jian et al. 2014b). Stored canola seeds are sensitive to elevated temperature because of a risk of the heat damage and ultimately, loss of quality and quantity (Jian et al. 2014b; Pronyk et al. 2004; Sun et al. 2014a). The heat damage might change the seed colour, such as the increase of yellow seeds. It is not known whether different pre-harvest treatments such as straight cutting, swathing, or desiccant application can affect the safe storage time of seeds.

Oilseed kernels infected by microflora have a reduced ability to germinate (White and Jayas 1991; White et al. 1982a), and pre-harvest treatments may influence the vigour of stored seeds. Therefore, seed germination is one of the most sensitive factors that can be used to monitor the quality of stored seeds. The invisible mould infection is another parameter that is used in evaluating grain deterioration as the mould growth will consume the dry mass of stored seeds and will produce heat and water via the respiration of the mould (White et al. 1982b). Deteriorated seeds also change their biochemical compositions (Tipples 1995). In this case, free fatty acids (FAV) are produced from hydrolyzed triglycerides. Therefore, germination, mould infection, and FAV are important storage parameters that are used to evaluate the storability of oilseeds (Tipples 1995; White et al. 1982c).

During storage, the relative humidity (ERH) of air in stored grain at a given temperature (T) equilibrates with the grain moisture content (EMC), and vice versa. The relationship among the ERH and EMC is usually termed as EMC – ERH relationship or desorption/sorption isotherm. The EMC-ERH relationships for canola and rapeseed were developed 20 y ago (Yang and Cenkowski 1994). In the last 25 y, new varieties (such as high oil content canola) were developed (Jian et al. 2013). The chemical composition of

oilseeds significantly influences sorption and desorption isotherms. The high oil content canola usually has about ten percentage points higher oil content than that of older cultivars (Sun et al. 2014b). This high percentage of oil will affect its desorption and sorption isotherms. Using the wrong EMC-ERH relationship to estimate canola EMC during drying or aeration could result in wet or over dried canola, which may result in spoiled oilseeds during storage. It is not known whether this relationship developed 25 y ago can still be suitable for use for the new varieties of the high oil content canola for the practice of drying and storage.

The objectives of this study were to evaluate the safe storage time of canola seeds harvested by the following five methods: swathed (referred to as SW), Glyphosate application + straight cut (referred to as GL), Heat and Glyphosate application + straight cut (referred to as HG), Reglone application + straight cut (referred to as RE), and natural ripening + straight cut (referred to as NR). The safe storage times of canola seeds with the different pre-harvest treatments were evaluated by determining seed moisture content, germination, FAV, yellow seed count, and invisible mould. At the same time, the EMC – ERH relationship for the temperature range between 20 to 35°C was evaluated, and the measured EMCs were compared with the EMCs predicted by the equations recommended by the ASABE standard (ASABE, 2016a).

## MATERIALS AND METHODS

### Pre-harvest treatment and preparation of canola seeds

The method of field treatment and preparation of canola seeds were the same as reported by Jian et al. (2019). Also, experimental procedures are similar to those reported earlier (Mills et al. 1978; Schroth et al. 1998; Sun et al. 2014b). To avoid repetition and enhance the readability of this manuscript, the method and preparation procedure of seeds and experimental procedures are summarised as follows. The application of pre-harvest aids (Glyphosate, Heat LQ, Heat LQ + Glyphosate, and Reglone) and harvest time was decided based on the farmer's experience and recommendations from the Canola Council of Canada (Canola Council of Canada, 2019) and the pre-harvest aid manufacturer (Bayer CropScience Inc., Morrisville, NC, USA). About 600 kg of canola seeds were collected from each field treatment. After three mo storage under room condition ( $25 \pm 3^\circ\text{C}$ ) inside tote bags, the 600 kg seeds were cleaned using a hand sieve (No.14, 5.56 mm openings), bagged in double-layer plastic bags, and stored at  $5 \pm 1^\circ\text{C}$  before use. The moisture content of the canola seeds at the harvest was  $10.5 \pm 0.1\%$  and was adjusted to  $6.5 \pm 0.1$ ,  $7.5 \pm 0.1$ ,  $10.5 \pm 0.1$  (by drying at room condition) and  $15.5 \pm 0.1\%$  (by adding water) before storage. These adjusted MCs were reported as the initial MCs in this article. Canola MC was determined by drying triplicate samples at  $130^\circ\text{C}$  for 4 h (ASABE, 2016b).

### Storage conditions

Canola seeds (6 kg) for each pre-harvest treatment and each initial MC were evenly divided into three equal portions, and each portion was bagged inside one cotton bag. The

cloth material of the cotton bag had less than 1.168 mm openings. The desired RH was achieved by using four saturated salt solutions:  $\text{Mg}(\text{NO}_3)_2$ ,  $\text{NaNO}_2$ ,  $\text{NaCl}$ , and  $\text{KNO}_3$  to produce approximately 52, 63, 75 and 93% RH, respectively (Winston and Bates 1960). About 2 L of each salt solution was poured into a 20 L pail. Inside each pail, a mesh plastic plate was located on top of three PVC pipes (about 8 cm diameter and 10 cm high), and half of each PVC pipe was above the salt solution. Inside each pail, three cotton bags with the canola seeds at the same pre-harvest treatment and the same initial MC (three replicates for each pre-harvest treatment) were located on the mesh plastic plate. The seeds with MCs of 6.5, 7.5, 10.5, and 15.5% were kept at 52, 63, 75, and 93% RH, respectively, during the test period. There were about 10 cm gaps between two bags, and the pails were covered by lids, to ensure that the canola seeds inside each bag would equilibrate with the RH produced by the salt solution inside the pail. The lids were opened and closed for about 1 min every 1 wk during the sampling period. The pails were stored inside four environmental chambers (Conviroon CMP3244, Controlled Environments Ltd., Winnipeg, MB, Canada) and temperatures of the chambers were set at  $20 \pm 1$ ,  $25 \pm 1$ ,  $30 \pm 1$ , and  $35 \pm 1^\circ\text{C}$ . The following storage conditions were tested for each of the five pre-harvest treatments:  $25^\circ\text{C}$  at 52, 63, and 93% RH; and 75% RH at 20, 25, 30, and  $35^\circ\text{C}$  to decrease workload. There were three replicates for each tested condition.

### Seed sampling and parameter determination

Every week, about 30 g of seeds from each cotton bag was taken to determine the MC, FAV, seed germination, green and yellow seed count, and invisible microflora. The seed MC was determined every 1 wk, and other parameters were determined every 2 or 3 wk. About 10 g seeds in the 30 g sample were used to determine the MC. After the determination of the MC, this dried sample was used to determine the FAV. Twenty-five seeds in the 30 g sample were used to determine the germination. Another 25 seeds in the 30 g sample were used to determine the invisible mould. Two hundred seeds in the 30 g sample were used to determine the yellow seed count. Experiments were stopped at 20 wk or terminated in less than 20 wk after the germination was less than 60%. There were three replicates for any measured parameter from three cotton bags at each storage condition studied.

**Invisible mould determination** To determine the invisible mould, 25 seeds were placed in a 9.0 cm diameter petri dish with a filter paper and 5 mL of 7.5% aqueous sodium chloride solution. After 7 d of incubation at room temperature, the invisible mould was identified under a dissecting microscope. The colour and shape of the microfloral colony were used to classify the mould species (Mills et al. 1978; Sun et al. 2014b). 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.

**FAV measurement** A Stein mill (M-2, Fred Stein Laboratories, Inc, Atchinson, KS) was used to grind the

dried sample. Five gram of the ground sample was rolled in a filter paper (No. 5 Whatman) and kept in a Goldfish Fat Extractor (Laboratory Construction Co, Kansas City, MO) for 6 hours of extraction. The extraction solvent was 30 mL of petroleum ether. After the evaporation of petroleum ether, 25 mL TAP solution (50% toluene and 50% ethanol with phenolphthalein indicator) was added to the extracted oil. A standardized KOH solution was used to titrate the solution until the appearance of a pale pink colour, and the FAV value was expressed as mg KOH/100 g dry sample (Schroth et al. 1998; Sun et al. 2014b).

**Germination measurement** Germination was determined by placing 25 seeds in a 9.0 cm diameter petri dish with a filter paper (No. 3 Whatman) and soaked with 5 mL of distilled water (AACC 1962). The seeds were incubated at room temperature ( $25 \pm 2^\circ\text{C}$ ), 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.

**Green and yellow seed count** Green and yellow seed counts were determined by following the Official Grain Grading Guide of Canadian Grain Commission (Canadian Grain Commission 2018). Canola seeds were placed on a plastic paddle ( $2 \times 10$  inch), and then masking tapes were used to keep 200 seeds on the paddle. A vinyl roller was used for crushing the seeds, and the number of seeds (on the tapes) with green and yellow colour was counted. The percentage of the yellow seeds was calculated and reported as the yellow seed count.

#### Data analysis

To evaluate the desorption properties and germination of canola seeds with different pre-harvest treatments, the means of canola MCs or germinations at the same storage condition, but different pre-harvest treatments were compared by conducting Paired t-test. To determine whether the equilibrium moisture content equations recommended by the ASABE standard (ASABE 2016a) can

be used to predict the EMC of the stored canola, the predicted EMC was compared with the MC measured at 3 wk by calculating the absolute difference between the predicted and measured MCs. The average absolute difference was calculated for each pre-harvest treatment. The maximum and minimum average absolute differences were reported in this article. To determine whether canola seeds with different pre-harvest treatments had different microfloral infections, Tukey's test was conducted to compare the percentages of fungi infection on the seeds kept at the same storage condition and for the same storage time but had different pre-harvest treatments.

## RESULTS AND DISCUSSION

### Moisture content

The canola seeds took 2 to 3 wk to reach their EMCs, and the initial MCs were higher than the EMCs of the canola under the storage conditions (Fig. 1). Therefore, canola seeds experienced water desorption at the beginning of storage. Some of the pails were not sealed well in the second week, so the MCs of canola seeds in some pails decreased and then increased after the sealing was improved (Fig. 1). This experimental error resulted in less than 0.5 percentage points of moisture content increase. The canola MCs after this increase was also lower than the initial MCs. Therefore, some of the seeds were not stored at constant moisture contents and had a less than 0.5 percentage point fluctuation.

All equations recommended by the ASABE (ASABE 2016a) could not predict the measured EMCs of canola seeds with different pre-harvest treatments (Fig. 2). At 75% RH, the best equation, which had the minimum value of the average absolute differences between the predicted and measured EMCs, was Eq. C for the variety of Tobin (ASABE, 2016a). The minimum and maximum average absolute differences between the EMCs measured and predicted by Eq. C for Tobin were 0.5 and 1.6 percentage points, respectively. At  $25^\circ\text{C}$ , the best equation was Eq. C

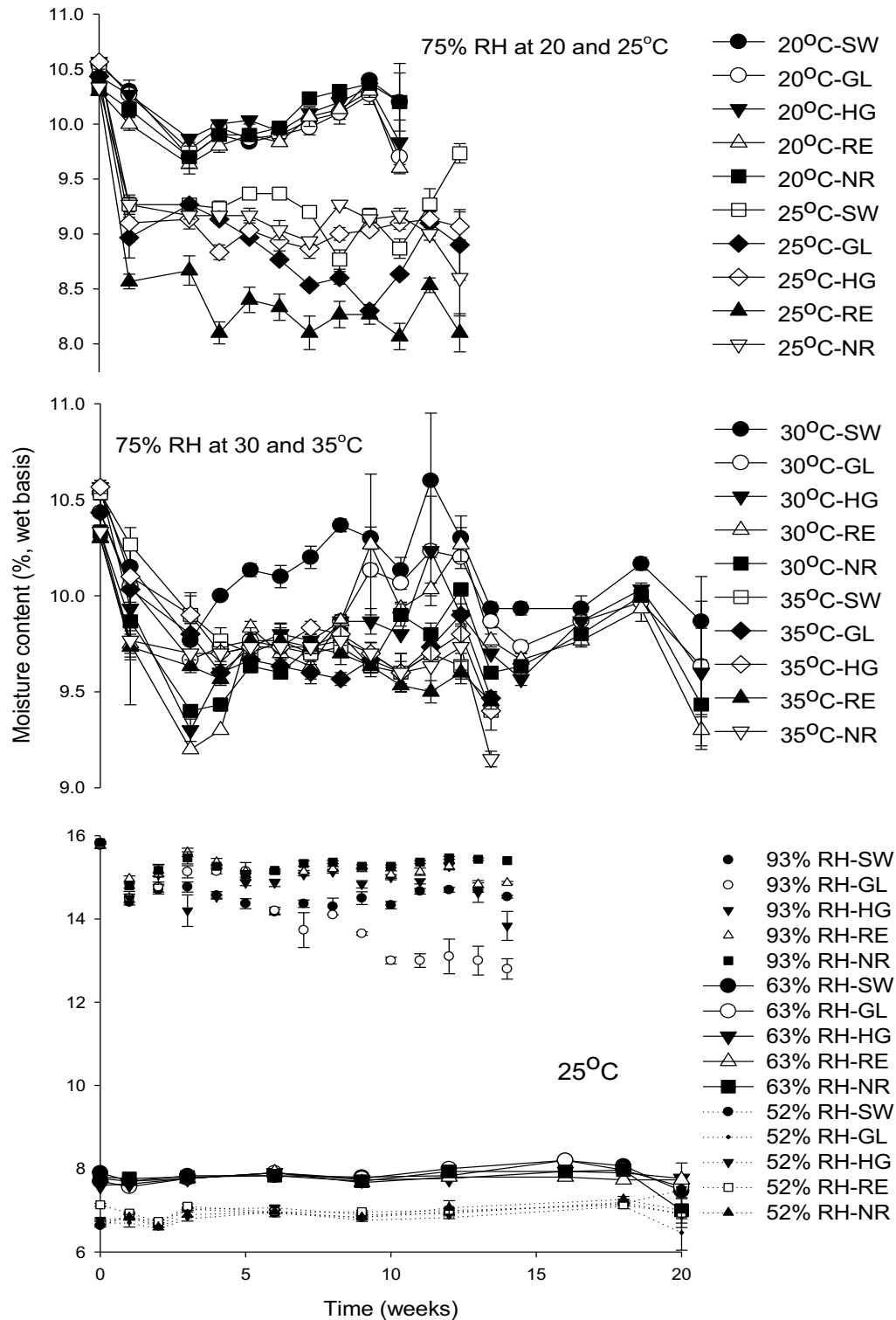
**Table 1. The p values of the Paired t-test to compare the moisture contents of canola with different pre-harvest treatments and under the same storage conditions.**

Pre-harvest treatment	Storage conditions							
	75% RH 20°C ‡	93% RH 25°C ‡	75% RH 25°C ‡	64% RH 25°C ‡	52% RH 25°C ‡	75% RH 30°C ‡	75% RH 35°C ‡	
SW-GL †	0.237	0.013*	0.001*	0.587	0.132	<0.001*	0.205	
SW-HG †	0.655	0.029*	0.035*	0.272	0.776	<0.001*	0.459	
SW-RE †	0.065	<0.001*	<0.001*	0.239	0.695	<0.001*	0.040*	
SW-NR †	0.756	<0.001*	0.213	0.124	0.495	<0.001*	0.107	
GL-HG †	<0.001	<0.001*	0.041*	0.344	0.002*	0.021*	0.036*	
GL-RE †	0.098	<0.001*	<0.001*	0.341	0.003*	0.026*	0.197	
GL-NR †	0.231	<0.001*	0.051	0.328	0.035*	0.010*	0.478	
HG-RE †	<0.001	0.012*	<0.001*	0.674	0.218	0.334	0.004*	
HG-NR †	0.864	0.004*	0.581	0.768	0.529	0.679	0.010*	
RE-NR †	0.022*	0.020*	<0.001*	0.879	0.196	0.780	0.389	

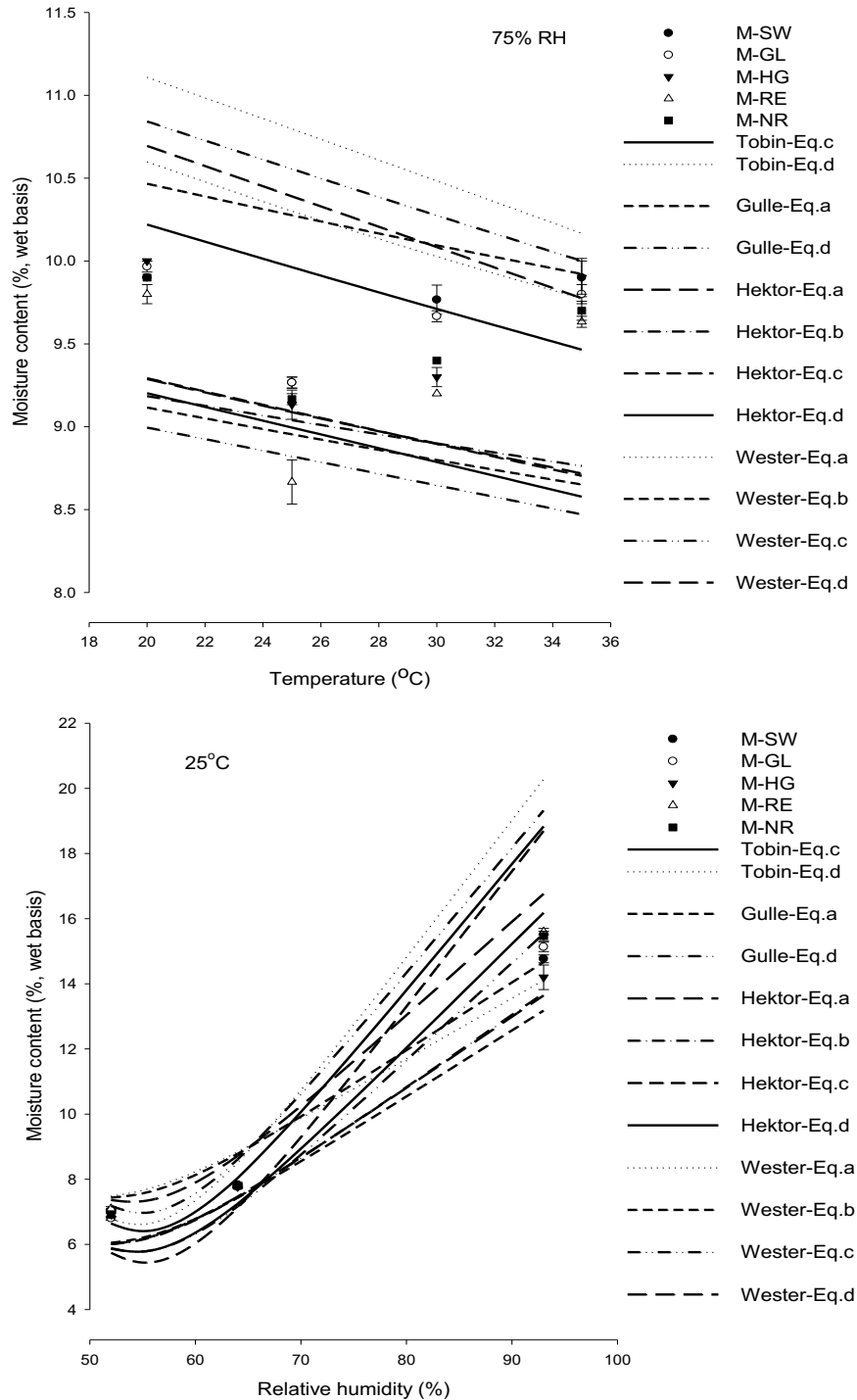
† Comparison among pre-harvest treatments (Paired t-test). SW, GL, HG, RE, and NR are the canola seeds treated by different pre-harvested treatments: swathed, Glyphosate application + straight cut (SC), Heat and Glyphosate application + SC, Reglone application + SC, and natural ripening + SC, respectively.

‡ 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.



**Fig. 1.** The moisture content of canola with different pre-harvest treatments under different temperatures at 75% relative humidity (top and middle) or different relative humidities at 25°C (bottom). In the graph, SW, GL, HG, RE, and NR are the canola seeds treated by different pre-harvested treatments: swathed, Glyphosate application + straight cut (SC), Heat and Glyphosate application + SC, Reglone application + SC, and natural ripening + SC, respectively.



**Fig. 2. Predicted and measured equilibrium moisture contents of canola with different pre-harvest treatments under different temperatures at 75% relative humidity (top) or different relative humidities at 25°C (bottom). In the legend, M = measured canola moisture contents. Tobin, Gulle, Hektor, Wester are the canola varieties used by the ASABE standard (ASABE 2016a) to develop the desorption equations. The lines are the predicted equilibrium moisture contents. SW, GL, HG, RE, and NR are the canola seeds treated by different pre-harvested treatments: swathed, Glyphosate application + straight cut (SC), Heat and Glyphosate application + SC, Reglone application + SC, and natural ripening + SC, respectively.**

**Table 2. Percentage of invisible mould infection on canola seeds with different pre-harvest treatments and under the same storage conditions.**

Storage condition	Storage time (wk) ‡	Microfloral species †				
		<i>Alternaria alternata</i>	<i>Aspergillus flavus</i>	<i>Aspergillus candidus</i>	<i>Aspergillus wentii</i>	<i>Penicillium spp.</i>
20°C, 75% RH	2	* 33.3 – 0.0	* 24.4 – 4.0	* 2.3 – 0.0		
	6		14.9 ± 1.9	15.2 ± 1.5		* 1.3 – 0.0
	14		18.7 ± 4.0	40.8 ± 4.8		* 4.0 – 0.0
25°C, 93% RH	2		* 42.7 – 13.3	* 18.7 – 0.0		
	6		85.6 ± 2.9	23.2 ± 2.4		0.8 ± 0.4
	14		93.1 ± 2.0	48.2 ± 7.1		2.4 ± 1.3
25°C, 75% RH	2	* 21.3 – 0.0	* 36.0 – 9.3	* 12.0 – 0.0		
	6		* 98.7 – 61.3	19.2 ± 3.0	1.6 ± 0.8	1.6 ± 0.9
	14		* 100.0 – 78.0	48.6 ± 5.9		5.1 ± 2.9
25°C, 64% RH	2	* 24.0 – 0.0	* 33.3 – 4.0	* 6.7 – 0.0		
	6		53.9 ± 3.9	14.9 ± 2.4		4.8 ± 0.8
	14		78.1 ± 4.0	40.5 ± 6.4		14.4 ± 2.4
25°C, 52% RH	2	* 33.3 – 0.0	19.7 ± 0.9	* 6.7 – 0.0		
	6		61.4 ± 4.1	11.1 ± 2.2	0.3 ± 0.3	5.1 ± 0.9
	14		81.3 ± 3.0	* 64.0 – 10.7		15.5 ± 2.6
30°C, 75% RH	2	* 10.7 – 0.0	10.7 ± 2.2			5.1 ± 1.0
	6		* 56.0 – 13.3	17.1 ± 2.0		5.3 ± 1.4
	14		* 100.0 – 18.7	51.2 ± 6.0		10.4 ± 2.0
35°C, 75% RH	2	* 5.3 – 0.0	* 44.0 – 0.0			4.2 ± 0.6
	6		84.5 ± 3.0	3.2 ± 1.0		2.4 ± 0.8
	14		94.9 ± 1.5	9.6 ± 2.9		10.9 ± 1.0

† Tukey's test was conducted to compare the percentages of the invisible mould of the fungi species infecting canola seeds with different pre-harvest treatments at the same storage condition (df = 14 for all Tukey's tests). If  $p < 0.05^*$ , the range of the percentages (maximum mean in the five pre-harvest treatments – minimum mean in the five pre-harvest treatments) is presented, otherwise the mean ± SE of the percentages of the five different pre-harvest treatments is presented. Empty cells indicate no mould.

‡ The data at 8, 12, 16 wk are not presented because these data were similar to that at 6 or 14 wk.

for the variety of Hector (ASABE 2016a) and the values of the minimum and maximum average absolute differences were 1.4 and 5.2 percentage points, respectively.

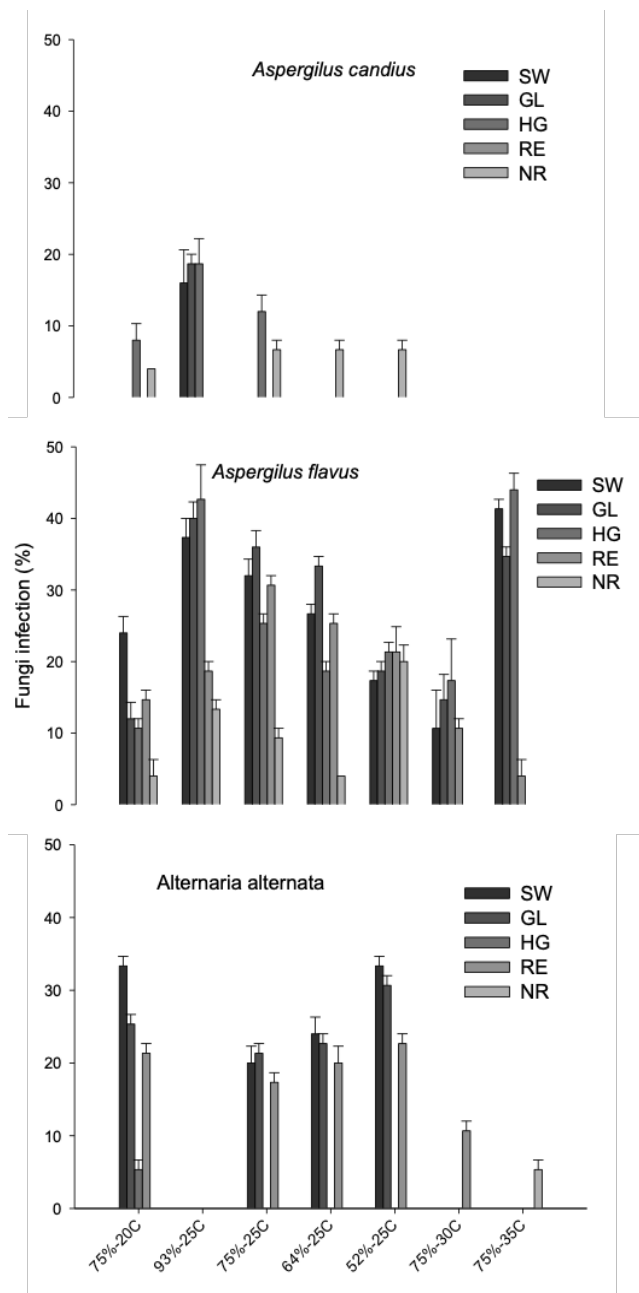
The main reason for the large absolute differences at 25°C was that about half of these equations had a higher predicted EMC at 93% ERH (Fig. 2), and no equation could predict the measured EMCs when all the measured MCs were used. These prediction errors might be mainly caused by the physical and chemical (such as higher oil contents than that of Hector and Tobin) properties of new varieties. They would not be acceptable because canola with 10% MC under Canadian conditions could be safely stored for about four months, but canola with 11% MC could only be stored for less than four months (Sun et al. 2014b). The canola seeds with initial MC of 15.5% were prepared by adsorption, and the seeds with less than 10.5% MCs were prepared by desorption. During the storage, all the canola seeds were under desorption. All the desorption and adsorption equations recommended by the ABABE standard could not fit the measured MCs. Hysteresis of the seeds under the desorption condition might not be the reason causing this prediction error because the canola seeds were under desorption condition for more than 8 wk. Therefore, the equilibrium moisture content of new canola varieties should be re-developed. Different methods to evaluate the equilibrium moisture content would produce

different EMC at the same temperature and ERH (Jian et al. 2018). To safely store canola seeds, therefore, the EMC-ERH isotherms should be evaluated at grain drying, aeration, and storage conditions.

#### Invisible mould

*Alternaria alternata* was only found on the seeds in 2 wk, and there were significant differences among pre-harvest treatments (Table 2). This significant difference was caused by the 0 or low infection on NR seeds. *Alternaria alternata* was not found in the seeds stored at 25°C + 93% RH. *Alternaria alternata* is a pre-harvest mould, and the occurrence of this fungal indicated that the seeds are stored at safe storage conditions (Pronyk et al. 2004). Therefore, *A. alternata* was gradually replaced by storage microflora as the increase of storage time, and the NR seeds or the seeds stored at the higher RH had been infected by storage microflora in less than 2 wk.

*Aspergillus wentii* rarely infected the seeds, and there was no significant difference among the seeds with different pre-harvest treatments (Table 2). The common fungal species were *Penicillium spp.*, *A. flavus*, and *A. candidus* Link (Table 2), which was consistent with the literature (Mills and Sinha 1980; Pronyk et al. 2004; Sun et al. 2014b). These three common species infected the seeds in the entire storage period, and there was no significant difference among different pre-harvest treatments when storage time



**Fig. 3. Fungi infection (%) on canola seeds with different pre-harvest treatments at different storage conditions. In the graph, the number before "C" and after "-" is the temperature (°C), the number before "%" is the relative humidity (%). SW, GL, HG, RE, and NR are the canola seeds treated by different pre-harvested treatments: swathed, Glyphosate application + straight cut (SC), Heat and Glyphosate application + SC, Reglone application + SC, and natural ripening + SC, respectively.**

was  $\geq 4$  wk in 80% cases. In contrast, the significant difference occurred in the 2 wk storage period for *A. flavus* and *A. candidus* in 86% cases. Final microflora counts showed that seeds at  $\geq 75\%$  RH and  $\geq 25^\circ\text{C}$  had nearly 100% infection by *A. flavus*. This is consistent with the literature (Pronyk et al. 2004; Sun et al. 2014b). For *A. candidus* at  $20^\circ\text{C}$  or  $25^\circ\text{C} + 64$  or  $52\%$  RH, this significant difference in the 2 wk storage period was caused by the 0 infections on SW, GL, HG, and RE seeds, while NR seeds had a less than 10% infection (Fig. 3, Table 2). For *A. candidus* at  $25^\circ\text{C} + 93$  or  $75\%$  RH, this significant difference in the 2 wk storage period was caused by the 0 infections on NR or GL seeds, while the seeds with other pre-harvest treatments had a less than 18.7% infection (Fig. 3, Table 2). At 2 wk of the storage time, the dominant fungal species was *A. flavus*, and it also had a higher infection at  $35^\circ\text{C}$  and  $93\%$  RH for any pre-harvest treatment except the NR canola (Fig. 3). The NR seeds had 0 or lower *A. flavus* infection at  $35^\circ\text{C}$  and  $93\%$  RH storage conditions. Therefore, different pre-harvest treatments had different fungal species with different initial infections. Generally speaking, NR seeds had the lowest initial fungal infection, SW seeds had the highest initial fungal infection, and other field treated seeds had in between initial fungal infection. However, these differences at the beginning of the storage did not influence the fungal infection in the storage period.

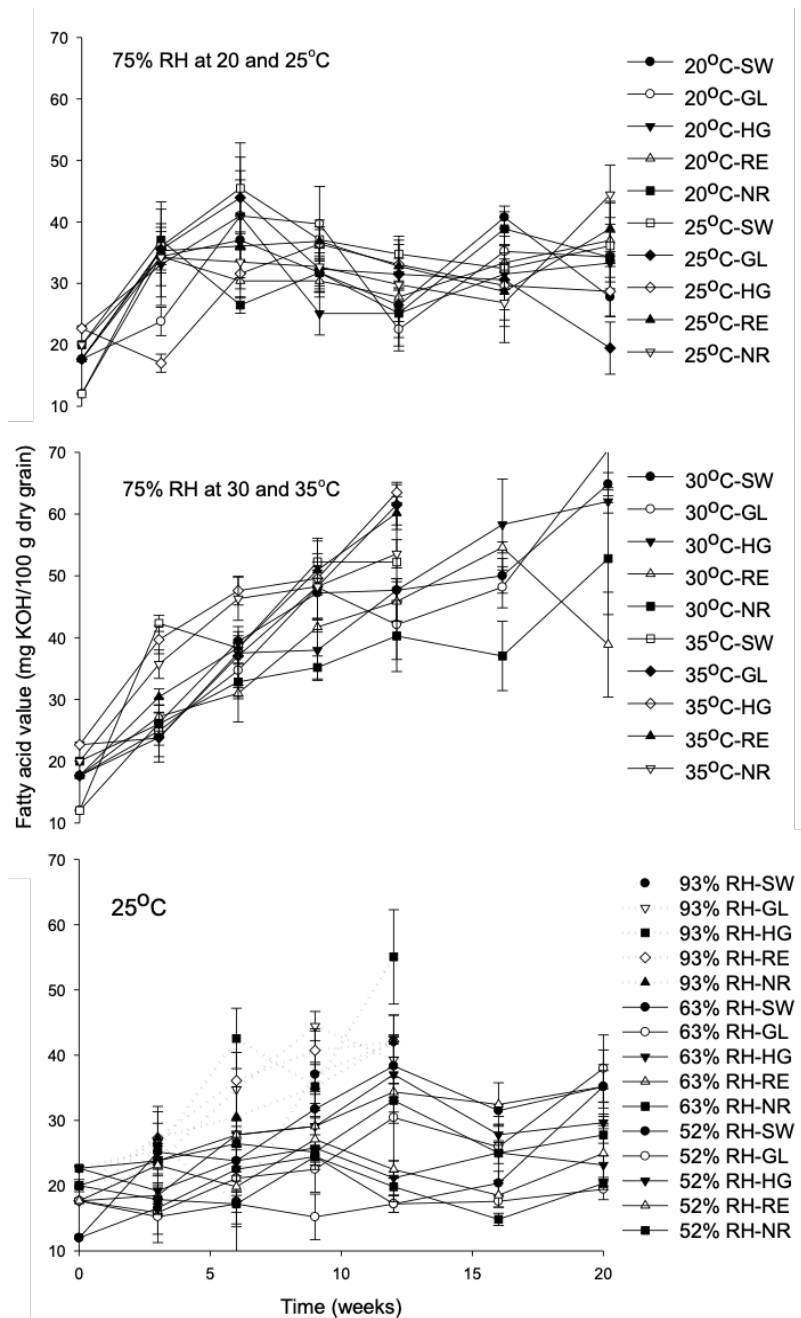
#### Fatty acid value

The initial FAV was about 20 mg KOH/100 g dry grain (Fig. 4), and there was no significant difference among the different pre-harvest treatments (Tukey test,  $F = 2.504$ ,  $p = 0.109$ ,  $df = 14$ ). The FAV gradually increased with the increase of time at any storage condition, but the seeds stored at higher temperatures or RH had a faster rise (Fig. 4). This initial FAV and the trend of the FAV change were the same as that reported in the literature (Everatt et al. 2014; Mills and Sinha 1980; Pronyk et al. 2004; Sun et al. 2014b). The canola seeds with different pre-harvest treatments had no significantly different FAV during the stored period (Paired t-test, all  $t < 1.330$ ,  $p > 0.254$ ), and there was also no significant difference at any storage time (Tukey test, all  $F < 1.729$ ,  $p > 0.220$ ,  $df = 14$ ). Therefore, different pre-harvest treatments did not influence the FAV of the stored canola. Free fatty acids are produced by the enzymatic activity of fungi consuming the seed (Tipples 1995). The conclusion of no significant difference of FAV indicated that different initial fungal infections of seeds with different pre-harvest treatments did not significantly influence the FAV production.

#### Germination

The initial germination of the canola was higher than 97% except for the SW seeds. The SW seeds had a significantly lower initial germination ( $88.0 \pm 1.3\%$ ) than any canola with different pre-harvest treatments (Student t-test, all  $t > 3.807$ ,  $P < 0.007$ ). Still, the germination of SW seeds increased after 5 wk. The sampling error might cause this initial low germination. The canola seeds with different pre-harvest treatments had no significantly different germination during the stored period (Paired t-test, all  $T < 0.974$ ,  $p > 0.311$ ).

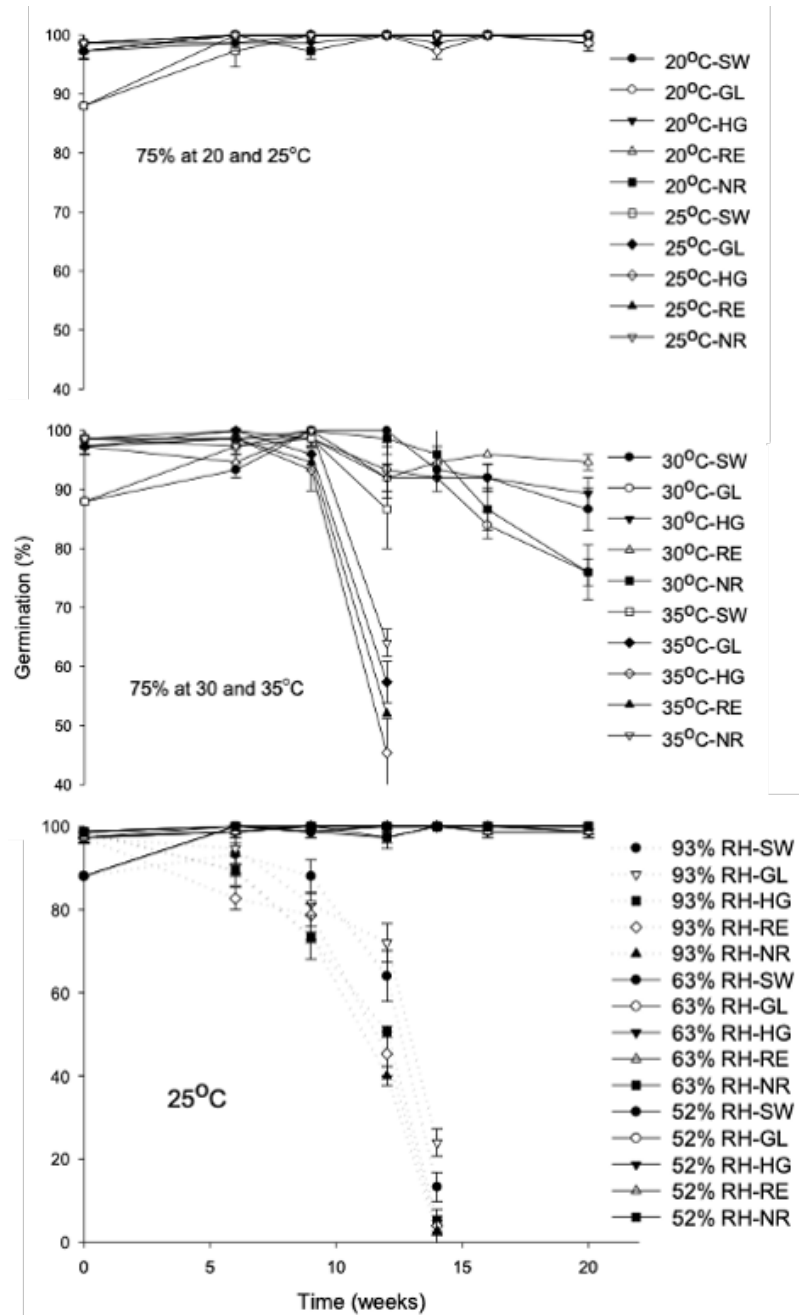




**Fig. 4. The fatty acid value of canola with different pre-harvest treatments under different temperatures at 75% relative humidity (top and middle) or different relative humidities at 25°C (bottom). In the graph, SW, GL, HG, RE, and NR are the canola seeds treated by different pre-harvested treatments: swathed, Glyphosate application + straight cut (SC), Heat and Glyphosate application + SC, Reglone application + SC, and natural ripening + SC, respectively.**

except the SW seeds stored at 35°C + 75% RH for 12 wk (Fig. 5), and this trend was the same as in the published report (Sun et al. 2014b). At 93% RH or 35°C, the rapid germination loss was associated with the increase of *A. flavus* and *A. candidus* (Table 2). This result indicated the canola with different pre-harvest treatments had the same trend of germination losses. The SW canola at 12 wk of storage time and stored at 35°C + 75% RH had significantly

higher germination ( $\geq 86.7\%$ ) than the canola with other pre-harvest treatments and stored at the same condition (Student t-test, all  $t > 3.213$ ,  $p < 0.033$ ). This significant difference did not hold at different temperatures or RHs (Fig. 5). This high germination of SW seeds might relate to the physiological change of canola exposed to different pre-harvest treatments (Jordan and Donaldson 1996; Syngenta Canada Inc. 2016).



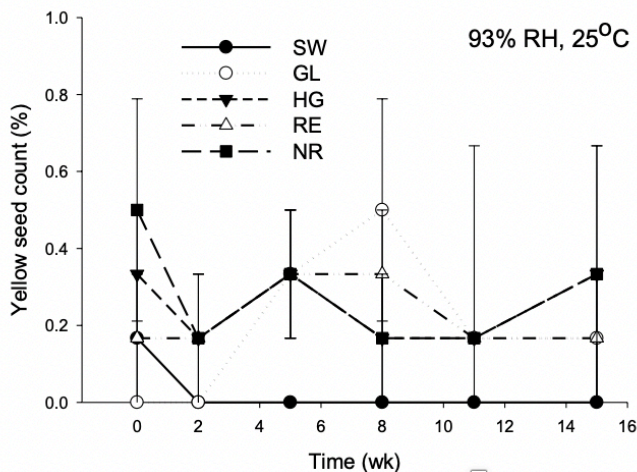
**Fig. 5. Germination of canola with different pre-harvest treatments under different temperatures at 75% relative humidity (top and middle) or different relative humidities at 25°C (bottom). In the graph, SW, GL, HG, RE, and NR are the canola seeds treated by different pre-harvested treatments: swathed, Glyphosate application + straight cut (SC), Heat and Glyphosate application + SC, Reglone application + SC, and natural ripening + SC, respectively.**

#### Yellow seed count

Green seed count was zero for all of the samples. The initial yellow seed count was  $0.18 \pm 0.05\%$ , and 12.4% of samples had yellow seeds. This initial yellow seed count was an indication of some immature seeds (Cenkowski et al. 1993; Cenkowski et al. 1989a). The yellow seed count was zero in the seeds stored at any storage condition after 8 wk except at 25°C + 93% RH condition. Under low RHs or

temperatures, the yellow seed count became zero in less than 3 wk. Therefore, yellow seed count could decrease under safe storage conditions, and microfloral infection at high relative humidities might be the main factor causing the increase of yellow seed count.

Under the highest RH condition, the yellow seed count did not decrease with the storage time except the SW canola (Fig. 6). The yellow seed count of the SW canola decreased



**Fig. 6. Yellow seed count (%) of the canola seeds with different pre-harvest treatments and stored at 93% RH + 25°C. In the graph, SW, GL, HG, RE, and NR are the canola seeds treated by different pre-harvested treatments: swathed, Glyphosate application + straight cut (SC), Heat and Glyphosate application + SC, Reglone application + SC, and natural ripening + SC, respectively.**

to zero at 5 wk at the highest RH condition. There were no significant differences among the other four pre-harvest treatments (Tukey's test at each storage time, all  $F \leq 0.133$ ,  $p \geq 0.937$ ,  $df = 11$ ). About 20 to 47% of samples had yellow seeds with these four pre-harvest treatments, and this percentage did not increase or decrease with the increase of storage time. Therefore, SW canola might have a higher resistance to damage from microfloral infection.

This study found the SW seeds had: 1) the highest initial fungal infection, 2) the highest germination at 35°C in 12 wk, and 3) the lowest or decreased yellow seed count at 93% RH among the different field treated seeds. Fungal growth inside seed cells or substances secreted by the fungi could kill seed germs (Hassan et al., 2013); Therefore, SW seeds might have a higher resistance to fungal development than other field treated seeds. The reason for this high resistance would be worth further investigation.

This study found canola seeds with different pre-harvest treatments and stored at  $\leq 25^\circ\text{C}$  or  $< 75\%$  RH did not have different germinations, FAV, and microfloral infection after 3 wk. Therefore, canola seeds with different pre-harvest treatments could be safely stored at  $\leq 8\%$  moisture content under Canadian conditions, and the safe storage time of the seeds treated by Glyphosate, Heat, or Reglone should be the same as the swathed seeds. However, the spoilage rate of canola with different pre-harvest treatments at the storage condition of high temperature and/or a high relative humidity was different.

## CONCLUSIONS

1. None of the equations recommended by the ASABE standard could predict the measured EMCs of canola exposed to different pre-harvest treatments. The minimum average absolute difference was larger than 0.5 percentage points when any one of these equations was used.
2. The swathed canola at 12 wk of storage time and stored at  $35^\circ\text{C} + 75\%$  RH had significantly higher germination ( $\geq 86.7\%$ ) than the canola with other pre-harvest treatments and stored at the same condition.
3. Different pre-harvest treatments did not affect the FAV of the stored canola.
4. Different pre-harvest treatments might have different fungal species with different initial infections. However, these differences at the beginning of the storage did not affect the fungal infection in the storage period.
5. Yellow seed count could decrease under safe storage conditions. At 93% RH, the yellow seed count increased with the storage time except for the SW canola. The yellow seed count of the swathed canola decreased to zero at 5 wk.

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