Frost damage to canola (*Brassica napus* L.) during reproductive phase in a controlled environment

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ABSTRACT

This study evaluated frost-related damage and benefits of cold acclimation during the reproductive phase in canola. Plants were exposed to cold acclimation and frost simulation at the start of flowering, during full flowering, and at the start of grain filling. The following were evaluated: plant mortality, number of viable ovules and silique abortion, floral buds and flowers, number of silique grains, total plant dry matter, number of siliques, total silique abortion, and grain dry matter. Frost caused death in non-acclimated plants and increased abortion in siliques, floral buds, and flowers, thus reducing silique and grain dry matter content. Frost mostly prevented the development of embryos in the siliques, but viable ovules were found in the floral buds and flowers. During their reproductive phase, canola plants have only a limited ability to increase their tolerance to frost damage. Nonetheless, siliques originating from the floral buds and open flowers contained a higher number of grains than siliques after frost exposure. Therefore, from the start of flowering to the start of grain filling, floral buds and flowers are responsible for producing most of the grain dry matter when there is frost occurrence.

Introduction

Canola (*Brassica napus* L.) exhibits good cold adaptability, especially when cold-acclimated. Cold acclimation is a process of exposure to low but non-freezing air temperatures that increases freezing tolerance (Fowler et al., 1999; Thomashow, 1999) that results in a reduced water potential in plant tissues (Gusta et al., 2004), since accumulated sugar in the vacuoles (Fujikawa et al., 1999), modified membrane composition, and a greater accumulation of unsaturated compared with saturated

fatty acids, allow greater membrane fluidity during freezing, thus reducing membrane damage (Ouellet et al., 2001; Pearce, 2001).

Cold acclimation is a quantitative multigenic process encompassing biochemical and structural changes that strongly affect plant physiology (Gusta et al., 2004; Levitt, 1972; Rapacz, 1999), including cell membranes, the first sites to be damaged in frost-related freezing events (Levitt, 1972). Changes due to cold acclimation promote stabilization of cell membranes, due an increased proportion of unsaturated and polyunsaturated fatty

acids in the lipid bilayers, resulting in greater membrane fluidity, thus ensuring the metabolic and structural functionality of cells at low air temperatures. Moreover, acclimation processes activate mechanisms that allow the full activity of enzymes associated with cell membranes during frost events (Uemura et al., 2003; Vogg et al., 1998). Acclimation also promotes the cytoplasmic accumulation of low molecular weight solutes, such as sugars and proline. Sugar accumulation increases cellular osmotic potential and consequently decreases the freezing point of cytoplasm, preventing the formation of intracellular ice crystals (Burg & Ferris, 2008; Ouellet & Charron, 2013). In addition, an increased cytoplasmic osmotic potential may reduce the rate of water displacement from the cytoplasm to intercellular spaces during frost occurrence, which causes formation of extracellular ice crystals, and prevent damage caused by cell desiccation. During frost, the presence of ice crystals in the intercellular spaces favors the transfer of water from the cell interior to exterior, due to the lower water vapor pressure on the ice surface than the liquid water vapor pressure in the cytoplasm. Solute accumulation in the cytoplasm as a result of acclimation leads to a reduced cytoplasmic osmotic potential, which disfavors the displacement of water from the cytoplasm to extracellular spaces during frost days (Levitt, 1980; Pearce, 2001).

However, in the absence of cold-acclimation, frost can cause severe damage during anthesis and grain filling of canola (Dalmago et al., 2010; Gusta et al., 2004; Hawkins et al., 2002; McClinchey and Kott, 2008; Rapacz, 1999; Rapacz et al., 2001). Lardon & Triboi-Blondel (1995) verified that frost-related plant death after anthesis is unusual in field cultivation; frost damage during the reproductive phases mainly concerns flower, silique, ovule, and embryo abortion, and reduced grain quality reduction due to chlorophyll retention (Lardon & Triboi-Blondel, 1995; McClinchey & Kott, 2008; Wilen et al., 1994). Open flowers on the upper main stem exhibit a higher cold sensitivity in canola reproductive structures than closed floral buds and siliques, because of reduced photoassimilate availability associated with low air temperatures in the upper canopy (Lardon & Triboi-Blondel, 1995). In a controlled environment, air temperature of –4°C during canola anthesis reduces the number of siliques per plant and grains per silique, probably due to ovule death (Dalmago et al., 2010; Lardon & Triboi-Blondel, 1994).

Frost damage to canola flowers and siliques at different reproductive stages has not however been described, and the processes involved during reproduction, and a quantification of damage, remain unclear. The high variability in meteorological conditions in southern Brazil during the canola cultivation period, including frost occurrence, makes this an important area of study,

especially in the context of extemporaneous frost events not preceded by an acclimation period. Our objective was therefore to determine the damage to canola during reproduction caused by simulated frost in cold-acclimated and non-acclimated plants grown in a controlled environment.

Materials and Methods

Experimental design

The research was performed in Passo Fundo, Rio Grande do Sul, Brazil (28°15´S, 52°24´W and 687 m altitude) in year 2016. Two experiments were carried out under controlled conditions in growth chambers and glass greenhouse with an air temperature control system. In both experiments, canola plants were cultivated in growth chambers from sowing until the start of frost simulation. After the frost simulation, plants were placed in a greenhouse to complete the development cycle.

It was utilized completely randomized design with a factorial arrangement. Factors were 'acclimation' (plants with and without cold acclimation) and 'frost' (plants with and without frost simulation). Seeds were sown in 48 polyvinyl chloride (PVC) pots (diameter, 22 cm; height, 25 cm; capacity, 8 kg soil). The soil was obtained from horizon A of a soil classified as Rhodic Hapludox. Fertilization was undertaken to achieve a yield of 2.0 t ha^{-1} grains. Five seeds were sown per pot, and then thinned to two plants per pot when the plants presented two to three developed leaves. Each plant was considered a separate experimental unit because plants in the same pot could present with different developmental stages when the acclimation and frost treatments were applied. Irrigation was performed daily in order to maintain soil moisture levels. Moisture control was performed using a portable probe (Hydrosense II with HS2 display; Campbell Scientific, São Paulo, Brazil); the average soil moisture in the pots was 28 % for both experiments on the day that plants were exposed to frost. The developmental stage of each plant was evaluated every two days, and on the day that plants were submitted to frost simulation, using the phenological scale adopted by CETIOM (Centre Technique Interprofessionnel des Oléagineux et du Chanvre) (Iriarte & Valetti, 2008).

Plant genotypes and developmental cycles

In order to evaluate the effect of the treatment combinations (frost and acclimation) at different points in the canola reproductive period, genotypes with different development cycles were used: Hyola 433 (early cycle), Hyola 61 (medium cycle), and Hyola 76 (late cycle) in experiment 1; and Diamond (early cycle), and Hyola 61 (medium cycle) in experiment 2. This strategy ensured that, on the day of the frost simulation, the following

canola phenological stages were represented: separated floral buds (E), first open flowers (F1), main branch elongation and several open flowers (F2), petal fall and the first ten 2 cm long siliques (G1), and siliques ≤ 4 cm (G3). The plants were classified into three groups, based on which type of reproductive structure was predominant on the main stem: beginning of flowering (BF; stages E and F1); full flowering (FF; stage F2); and beginning of grain filling (GF; stages G1 and G3). Thus, 41, 24, and 31 plants (experiment 1), and 19, 18, and 59 plants (experiment 2), were placed in the BF, FF, and GF groups, respectively for each experiment. Proceeding the groups there was mixture of genotypes. It is possible that genotypes have different levels of frost tolerance but for first our objective it is identify the response of canola plants in different phases of the reproductive period.

Experimental conditions, cold acclimation and frost simulation

The growth chamber used for canola cultivation from sowing to frost simulation (growth chamber 1) had the following characteristics: 12 h light (daytime) and 12 h dark (nictoperiod), with photosynthetically active radiation (PAR) in the range of 230 to 250 µmol m^{-2} s^{-1}; mean daily air temperature inside the chamber of 14°C (daytime, 18°C; nictoperiod, 10°C); and air relative humidity maintained between 80 % and 100 % during both day and night. Another growth chamber (growth chamber 2) was used for plant acclimation and frost simulation, with a photoperiod, air relative humidity, and PAR equal to that used in growth chamber 1.

Before canola plants were exposed to the two cold acclimation regimes (with and without acclimation), treatments 1 and 2 were submitted to air temperature regime of 20°C (daytime) and 16°C (nictoperiod) (daily mean of 18°C) for seven days. This thermal regime was adopted in both experiments with the objective of eliminating or reducing any cold acclimation level previously acquired by plants from the thermal regime adopted during growth (Rife & Zenaili, 2003). After this 'loss of acclimation' period, plants were exposed to the two cold acclimation regimes.

Cold acclimation began when the plants reached phenological stage E. This occurred at 75 and 70 days after sowing in experiments 1 and 2, respectively. The thermal regime for plant acclimation was based on a methodology adopted by Dalmago et al. (2010). Canola plants were submitted to air temperatures ranging from 2 to 8°C (nictoperiod), and 8 to 13°C (daytime), for three consecutive days. Air relative humidity was 50–60 % (daytime) and 80–100 % (nictoperiod). Non-acclimated plants were exposed to air temperature of 16°C (nictoperiod) and 20°C (daytime). The minimum air temperature of 16°C was adopted because cold acclimation starts at air temperatures < 15°C (Rapacz et al., 2001; Rife & Zeinali, 2003).

The frost simulation was performed after plant exposure to the acclimation regimes (with and without cold acclimation) in growth chamber 2, while plants without frost exposure were placed in growth chamber 1 (with and without cold acclimation). Soil moisture in all pots was assessed on the day of the frost simulation. Plants were submitted to a thermal regime of frost simulation for 48 h. The mean air temperature for the first 12 h was 5°C. It was then gradually reduced to –6°C in the subsequent 12 h. After reaching a minimum value of –6°C, the air temperature was gradually increased for 4 h, to 2°C, followed by a slower increase for 12 h, from 2 to 9°C. For the remaining period (8 h), the air temperature was increased from 9 to 11°C. At the end of the frost simulation thermal regime, the plants were transferred to growth chamber 1, with the rest of the plants that had not been exposed to frost. After the frost simulation, all plants remained in the growth chamber for a further seven days in an air temperature regime of 20°C (daytime) and 16°C in the night period (daily mean of 18°C). Thereafter, the plants were transferred to the greenhouse until physiological maturation was achieved.

Different types of reproductive structures were labeled before the frost simulation in order to identify the effect of frost and acclimation. In experiment 1, siliques of lengths < 2 cm, 2 to 4 cm, and > 4 cm were labeled. In experiment 2, closed floral buds, open flowers, and siliques of lengths < 2 cm and 2 to 4 cm were labeled. After the frost simulation, abortion of the labeled reproductive structures was evaluated biweekly. At harvest, the total number of each type of labeled structure that was developed in the siliques, and the number of grains per silique, was counted.

One day after the frost simulation, the number of normal ovules in open flowers and closed floral buds was counted in both experiments, based on the method of Lardon and Triboi-Blondel (1994), using an electronic microscope (SMZ-10; Nikon, Tokyo, Japan). The pistils were opened transversely with a scalpel, and the number of normal ovules counted. The number of closed floral buds and flowers ranged from 7 to 19 and 7 to 25, respectively, in the different treatments.

The number of siliques on the main stem (the trait most affected by frost), and total abortion of the reproductive structures were also recorded at harvest. The abortion of the reproductive structures was quantified by counting the number of inserts with peduncle remnants on the main stem. Abortion of reproductive structures on the main stem was calculated as a percentage based on the number of siliques and aborted reproductive structures. During grain-filling period (in the greenhouse), air temperature values of between 27 and 30°C were registred. However,

these air temperatures were only occurred on some dates, when all plants were at the grain-filling stage and were more tolerant of raised air temperatures than during anthesis, so the impact on the experimental results would have been minimal.

After physiological maturation, the plants were collected and separated into siliques and other parts, which were packed into paper bags and placed in an oven at 65°C until a constant weight was achieved. The siliques were then threshed, and grain dry matter and total plant dry matter using were quantify using a precision scale.

Data analysis

Analysis of variance was performed for each experiment, based on the plant development periods (BF, FF, and GF), for the following traits: total plant dry matter, grain dry matter, number of siliques, and abortion of reproductive structures. Also, analysis of variance was also performed for the following structures: the number of ovules in flowers and floral buds, abortion of different types of reproductive structures, and number of grains in siliques originating from these structures. One degree of freedom was removed from the error factor when treatments did not have the same number of replicates. Subsequently, the analysis of variance means were compared by a Tukey test at a 5 % error probability.

Results and Discussion

Plant mortality responses in different experimental conditions

The exposure of canola plants to frost (air temperature of –6°C) at three different points in the reproductive cycle (BF, FF, and GF) resulted in the death of plants that had not previously been cold-acclimated for three consecutive days before frost. Air temperature of –6°C resulted in the mortality of 67 %, 78 %, and 92 % of BF, FF, and GF plants in experiment 1, respectively, while in experiment 2, plant mortality at this air temperature was not verified, regard-less of the level of plant acclimation (Table 1).

In experiment 1, canola plant mortality during the reproductive stages corroborates the results of Lardon and Triboi-Blondel (1994), who confirm mortality in non-acclimated FF plants when exposed to air temperature lower than –3°C under controlled conditions. The survival of all acclimated frost-exposed plants demonstrated that canola can increase its freezing tolerance, even when exposed to short periods (3 d) of an air temperature regime with cold acclimation potential (Table 1), not only at the beginning of the development cycle but also during flowering (Dalmago et al., 2010). Rife & Zeinali (2003) affirm that canola plants in the vegetative phase present the maximum increase in cold tolerance in the first three days of acclimation.

It was not possible to determine accurately the reason why a temperature of –6°C caused mortality in non-acclimated plants in experiment 1, but not in experiment 2 (Table 1). Our hypothesis is that the plants in experiment 2 may have experienced water deficit, which possibly triggered responses similar to those for frost in cold-acclimated and non-acclimated plants (Silva et al., 2008). It is possible that differences in frost sensitivity among genotypes could have contributed to the contrasting plant mortality between the experiments, but evaluate genotypes it was not our objective. The literature also presents divergent information regarding frost sensitivity in non-acclimated canola plants during the reproductive period. As previously mentioned, Lardon and Triboi-Blondel (1995) report that an air temperature below –3°C can cause death in non-acclimated canola plants in their reproductive phase. Moreover, Rife & Zeinali (2003) have found that air temperature of –5.5°C results in canola plant mortality of 50 % at the beginning of the reproductive period, i.e., at an air temperature similar to that causing plant mortality in experiment 1 in our study. Dalmago et al. (2010) report that an air temperature of –6°C did not cause mortality in non-acclimated plants during their reproductive period, similar to our results for experiment 2. Although identifying damage levels at different air temperatures was not an objective of the present

Table 1 – Plant mortality of canola with and without cold acclimation, and with and without frost exposure, at the beginning of flowering, during full flowering, and at the beginning of grain filling, in experiments 1 and 2. Passo Fundo, RS – 2018.

	Beginning of Flowering		Full Flowering		Beginning of Grain Filling	
	Frost	**No Frost**	**Frost**	**No Frost**	**Frost**	**No Frost**
	%					
Experiment 1						
Acclimation	0	0	0	0	0	0
No Acclimation	67	0	78	0	92	0
Experiment 2						
Acclimation	0	0	0	0	0	0
No Acclimation	0	0	0	0	0	0

study, the differences in plant mortality in experiments 1 and 2, and information reported in the literature, indicate that there must be a lethal air temperature range for non--acclimated canola plants during their reproductive phases. This lethal range would have an below limit value close to –3°C, when the first plants die, and a lower limit value of upper –6°C. However, the existence of such a lethal air temperature range does not explain the different mortality levels between experiments 1 and 2.

Genes responsible for increasing cold tolerance are responsive to water stress, abscisic acid, and soil salinity. It has not been established how these different stimuli converge to induce the expression of these genes (Ouellet & Charron, 2013). It is possible that some of the stimuli, such as water stress, were in fact active in experiment 2, and promoted the activation of the genes associated with cold tolerance, thus increasing freezing tolerance in canola plants that had not been exposed to acclimation. Despite that nothing been observed in the experimental control and also monitoring of water of soil. Biochemical analysis would therefore be necessary for determining differences between acclimated and non-acclimated plants, in order to identify the acclimation level of canola plants on the day they were exposed to frost.

Response of dry matter content to experimental conditions

In experiment 1, frost exposure reduced the total dry matter of plants by 38 %, 49 % and 34 % in BF, FF, and GF, respectively (Table 2). In GF, frost had no significant effect on acclimated GF plants. As expected, no simple effect of acclimation was found on accumulation of total plant dry matter (Gusta et al., 2004), since the 3 d experimental period was too short to influence dry matter production in canola reproductive stages. However, acclimated canola plants presented a greater accumulation of dry matter under frost exposure (Dalmago et al., 2010), since acclimation prevented plant mortality and plants could continue their development until the end of the cycle. This response was confirmed by the accumulation of plant dry matter in experiment 2, with no plant mortality, and consequently no significant effect on the total accumulation of dry matter in plants exposed to frost (Table 2).

The effect of plant exposure to air temperature of –6°C was far more pronounced for grain dry matter from the main stem than for the total dry matter of plants (Tables 2 and 3). The quantity of canola plant dry matter was 39 %, 73 %, and 69 % lower in BF, FF, and GF, respectively, in plants exposed to frost. In BF plants, there was no interaction between acclimation and frost for grain dry matter in experiment 1 (Table 3). Frost damage to plants in experiment 2 were reduced in acclimation plants, since grain dry matter was 72 % higher than in plants exposed to frost without acclimation (Table 3). Therefore, the grain dry matter content was similar to plants that were not exposed to frost. In FF plants (experiment 1), acclimation reduced frost damage; however, these plants presented a lower grain dry matter content than plants not exposed to frost (Table 3). There was no effect of acclimation to reduced frost damage in experiment 2 (Table 3). In the GF group plants, frost caused a 69 % reduction in grain dry matter, and acclimation was not effective in reducing frost damage in either experiment (Table 3).

Effect of experimental conditions on reproductive structures

Frost was also responsible for the increased abortion of reproductive structures, at magnitudes of 43 %, 100 %, and 120 % in BF, FF, and GF, respectively, when compared with natural abortion in the plants not exposed to frost in experiment 1 (Table 4). In experiment 2, frost caused an increase in reproductive structure abortion of 82 %,

Table 2 – Total dry matter of canola plants with and without cold acclimation, and with and without frost exposure, at the beginning of flowering, during full flowering, and at the beginning of grain filling, in experiments 1 and 2. Passo Fundo, RS – 2018.

	Beginning of Flowering			Full Flowering			Beginning of Grain Filling		
	Frost	No Frost	Mean	Frost	No Frost	Mean	Frost	No Frost	Mean
	g plant⁻¹								
Experiment 1									
Acclimation	12.2	14.2	12.6 a	9.4	16.2	11.9 a	10.9 Aa	12.2 Aa	11.2
No Acclimation	8.1	19.5	10.5 a	6.5	14.1	8.9 b	6.5 Bb	12.7 Aa	7.5
Mean	10.4 B	16.8 A		7.8 B	15.2 A		8.2	12.5	
Experiment 2									
Acclimation	8.6	9.6	9.0 a	11.4	16.4	13.4 a	10.3	11.2	10.8 a
No Acclimation	9.2	12.2	10.7 a	11.5	14.2	12.9 a	8.9	12.6	10.8 a
Mean	8.8 A	10.8 A		11.5 A	15.3 A		9.5 A	11.9 A	

¹Means followed by the same letters (uppercase in rows and lowercase in columns) indicate no difference for each treatment according to a Tukey test (5 % error probability).

Table 3 – Grain dry matter of canola plants with and without cold acclimation, and with and without frost exposure, at the beginning of flowering, during full flowering, and at the beginning of grain filling, in experiments 1 and 2. Passo Fundo, RS – 2018.

	Beginning of Flowering			Full flowering			Beginning of Grain Filling		
	Frost	No Frost	Mean	Frost	No Frost	Mean	Frost	No Frost	Mean
	g plant⁻¹								
Experiment 1									
Acclimation	1.16	2.03	1.32 a	0.33 Ba	1.51 Ab	0.66	0.36	1.49	0.66 a
No Acclimation	0.16	1.19	0.38 b	0.03 Bb	1.88 Aa	0.18	0.0	1.35	0.34 b
Mean	0.71 B	1.61 A		0.06	1.69		0.17 B	1.42 A	
Experiment 2									
Acclimation	1.69 Aa	1.40 Aa	1.59	1.32	2.38	1.74 a	0.96	1.69	1.40 a
No Acclimation	0.98 Ba	2.33 Aa	1.65	0.87	2.19	1.53 a	0.74	1.65	1.20 a
Mean	1.44	1.86		1.14 B	2.28 A		0.83 B	1.67 A	

[1]Means followed by the same letters (uppercase in rows and lowercase in columns) indicate no difference for each treatment according to a Tukey test (5 % error probability).

55 % and 27 % in BF, FF and GF, respectively (Table 4). Plant acclimation had no significant effect on reducing the total abortion of reproductive structures in any of the three reproductive phases (Table 4). High variability in the natural abortion of reproductive structures (11 % to 46 %) is a characteristic of canola (Battisti et al., 2013). In our study, this variability, especially in the BF group, contributed to the fact that acclimation had no effect on reducing abortion.

The number of siliques on the main stem was directly influenced by frost-related abortion of reproductive structures, since a higher abortion rate results in fewer siliques (Table 5). The only exception occurred in BF plants in experiment 2, in which acclimation and frost had no effect on abortion of reproductive structures, and the number of siliques was similar in acclimated plants exposed and those not exposed to frost (Table 5). Dalmago et al. (2010) have also verified that frost occurrence at

the flowering stage caused a reduction in the number of siliques, due to the greater reproductive structure abortion rate in frost-exposed plants.

Evaluation of cold tolerance of different reproductive structures (Tables 6, 7, and 8), such as floral buds, flowers, and siliques of different lengths, showed that acclimation reduced the abortion of siliques of length ≥ 4 cm by 73 % in experiment 1 (Table 6). In experiment 2, there was no significant difference in the abortion of closed floral buds, flowers, siliques of length < 2 cm, and siliques of 2–4 cm length, when comparing plants exposed and not exposed to frost (Tables 7 and 8).

Although acclimation reduced the abortion of siliques of length > 4 cm (experiment 1), it did not increase the cold tolerance of grains in siliques of different lengths or in the ovules in floral buds and flowers (Tables 6, 7, 8 and 9). In experiment 1, frost caused a reduction of 51 %, 76 %, and 68 % in the number of viable flower ovules, and a reduction

Table 4 – Total abortion of reproductive structures in canola plants with and without cold acclimation, and with and without frost exposure, at the beginning of flowering, during full flowering, and at the beginning of grain filling, in experiments 1 and 2. Passo Fundo, RS – 2018.

	Beginning of Flowering			Full Flowering			Beginning of Grain Filling		
	Frost	No Frost	Mean	Frost	No Frost	Mean	Frost	No Frost	Mean
	%								
Experiment 1									
Acclimation	52.8	35.3	49.6 b	79.1 Ab	48.3 Ba	67.9	89.4 Ab	42.6 Ba	76.9
No Acclimation	92.9	63.6	86.8 a	97.3 Aa	39.8 Bb	79.6	99.1 Aa	43.2 Ba	85.1
Mean	71.0 A	49.5 B		89.3	44.1		94.4	42.9	
Experiment 2									
Acclimation	35.7 Aa	50.8 Aa	41.2	49.4	35.4	43.8 a	52.1	42.6	46.4 b
No Acclimation	49.7 Aa	27.3 Aa	38.5	55.5	31.3	43.4 a	60.1	46.7	53.4 a
Mean	40.8	39.1		51.8 A	33.4 B		56.9 A	44.7 B	

[1]Means followed by the same letters (uppercase in rows and lowercase in columns) indicate no difference for each treatment according to a Tukey test (5 % error probability).

Table 5 – Number of siliques in canola plants with and without cold acclimation, and with and without frost exposure, at the beginning of flowering, during full flowering, and at the beginning of grain filling, in experiments 1 and 2. Passo Fundo, RS – 2018.

	Beginning of Flowering			Full Flowering			Beginning of Grain Filling		
	Frost	No Frost	Mean	Frost	No Frost	Mean	Frost	No Frost	Mean
	siliques plant⁻¹								
Experiment 1									
Acclimation	32.3	50.4	35.3 a	12.8 B	35.2 A	21.0	7.4	37.2	15.3 a
No Acclimation	0.8	23.4	2.9 b	2.0 B	47.7 A	16.0	0.5	34.0	8.9 b
Mean	12.2 B	35.7A		6.7	41.5		3.8 B	35.6 A	
Experiment 2									
Acclimation	49.4 Aa	38.8 Ab	45.5	40.5	53.2	45.6 a	34.3	40.7	38.1 a
No Acclimation	39.0 Ba	69.5 Aa	54.2	32.2	62.2	47.2 a	27.2	42.6	34.9 a
Mean	45.6	54.1		37.2 B	57.7 A		30.1 B	41.7 A	

[1]Means followed by the same letters (uppercase in rows and lowercase in columns) indicate no difference for each treatment according to a Tukey test (5 % error probability).

Table 6 – Abortion of reproductive structures and number of grains per silique originating from the reproductive structures in canola plants with and without cold acclimation, and with and without frost exposure in experiment 1. Passo Fundo, RS – 2018.

	Siliques < 2 cm			Siliques 2–4 cm			Siliques > 4 cm		
	Frost	No Frost	Mean	Frost	No Frost	Mean	Frost	No Frost	Mean
	Abortion (%)								
Acclimation	63.8	27.5	53.1 b	58.8	10.0	42.6 b	25.0 Ab	0.0 Aa	13.9
No Acclimation	100.0	50.0	84.4 a	91.4	0.0	67.0 a	92.5 Aa	8.3 Ba	68.5
Mean	81.1 A	38.8 B		75.9 A	5.6 B		70.0	4.2	
	Number of grains per silique								
Acclimation	0.4 Ba	7.4 Aa	2.4	0.00	10.1	3.4 a	0.0	14.6	6.5 a
No Acclimation	0.00 Bb	4.1 Ab	1.3	0.1	8.2	2.2 a	0.0	14.1	4.0 b
Mean	0.2	5.8		0.1 B	9.3 A		0.0 B	14.3 A	

[1]Means followed by the same letters (uppercase in rows and lowercase in columns) indicate no difference for each treatment according to a Tukey test (5 % error probability).

Table 7 – Abortion of reproductive structures (buds and flowers) and number of grains per silique originating from the reproductive structures in canola plants with and without cold acclimation, and with and without frost exposure in experiment 2. Passo Fundo, RS – 2018.

	Buds			Flowers		
	Frost	No Frost	Mean	Frost	No Frost	Mean
	Abortion (%)					
Acclimation	6.4	5.0	5.8 a	8.3	2.8	6.4 a
No Acclimation	21.6	4.6	13.0 a	12.4	4.8	9.4 a
Mean	12.7 A	4.8 A		10.6 A	4.0 A	
	Number of grains per silique					
Acclimattion	5.3	6.4	5.8 a	9.9	14.7	14.6 a
No Acclimattion	7.7	7.9	7.8 a	9.2	6.3	8.0 a
Mean	6.5 A	7.2 A		9.5 A	9.8 A	

[1]Means followed by the same letters (uppercase in rows and lowercase in columns) indicate no difference for each treatment according to a Tukey test (5 % error probability).

of 41 %, 79 %, and 78 % in the number of viable flower bud ovules, in BF, FF, and GF, respectively (Table 9). In experiment 2, frost caused a 60 % reduction in the number of viable flower ovules in GF plants but had no effect on flowers of BF and FF plants (Table 9). Damaged ovules were withered and/or whitened (Lardon & Triboi-Blondel, 1994). Acclimation prevented plant death during the reproductive period (Table 1) but demonstrated a limited

Table 8 – Abortion of reproductive structures (siliques) and number of grains per silique originating from these structures in canola plants with and without cold acclimation, and with and without frost exposure in experiment 2. Passo Fundo, RS – 2018.

	Siliques < 2 cm			Siliques 2–4 cm		
	Frost	No Frost	Mean	Frost	No Frost	Mean
	Abortion (%)					
Acclimation	18.0	12.8	15.1 a	0.0	0.0	0.0 a
No Acclimation	12.4	7.9	10.1 a	0.0	5.8	3.6 a
Mean	10.2 A	14.8 A		0.0 A	2.7 A	
	Number of grains per silique					
Acclimation	1.3	6.8	4.4 a	1.7 Ba	21.6 Aa	13.6
No Acclimation	0.7	5.4	3.1 a	2.2 Ba	13.2 Ab	9.0
Mean	0.9 B	6.1 A		1.9	17.6	

[1]Means followed by the same letters (uppercase in rows and lowercase in columns) indicate no difference for each treatment according to a Tukey test (5 % error probability).

Table 9 – Number of viable ovules in closed and open flowers in canola plants with and without cold acclimation, and with and without frost exposure, at the beginning of flowering, during full flowering, and at the beginning of grain filling, in experiments 1 and 2. Passo Fundo, RS – 2018.

	Beginning of Flowering			Full Flowering			Beginning of Grain Filling		
	Frost	No Frost	Mean	Frost	No Frost	Mean	Frost	No Frost	Mean
	ovules flower[1]								
	Experiment 1 - Floral buds								
Acclimation	18.1	27.6	22.4 a	8.4 Ba	26.6 Aa	19.6	2.4 Ba	28.8 Aa	20.4
No Acclimation	15.0	29.1	22.4 a	3.6 Bb	27.5 Aa	17.2	8.47 Ba	26.6 Aa	19.7
Mean	16.8 B	28.3 A		5.7	27.1		6.2	27.7	
	Experiment 1 - Flowers								
Acclimation	16.7	29.4	23.0 a	14.0	28.8	23.0 a	9.6	28.7	20.0 a
No Acclimation	11.9	28.5	20.2 a	6.1	28.0	18.6 b	8.1	26.5	17.3 a
Mean	14.3 B	28.9 A		9.6 B	28.4 A		8.8 B	27.6 A	
	Experiment 2 - Flowers								
Acclimation	26.5	29.1	27.6 a	6.7	6.3	6.5 b	7.1	17.4	12.3 a
No Acclimation	13.1	19.6	16.7 b	15.3	11.2	13.2 a	1.9	4.8	3.4 b
Mean	22.2 A	24.8 A		10.1 A	8.7 A		4.5 B	11.1 A	

[1]Means followed by the same letters (uppercase in rows and lowercase in columns) indicate no difference for each treatment according to a Tukey test (5 % error probability).

capacity to increase cold tolerance in plant reproductive structures (Tables 6, 7, 8 and 9). This response corroborates with the results of Fowler et al. (1999), Mahfoozi et al. (2001), and Trischuk et al. (2014), which afirm that plants without vernalizing requirements have a limited ability to increase their cold tolerance in the reproductive period.

Frost did not reduce the number of grains in siliques originating from closed floral buds and open flowers but did reduce the number of grains when structures it was siliques (94 % in both experiments) (Tables 6, 7 and 8). This response indicated that ovules in floral buds and flowers are less susceptible to stress-induced cold damage than silique embryos, which are very sensitive to frost damage. Frost occurrence may inhibit embryo development when

content of water is over 80 %, since freezing of extracellular water causes significant cell dehydration and irreversible damage in the embryos (Johnson-Flanagan et al., 1991; Lardon & Triboi-Blondel, 1994).

Viable and frost-damaged ovules were found in the same pistil of flowers and floral buds, corroborating with Lardon and Triboi-Blondel (1994). This indicated that floral buds and flowers can develop into productive siliques, even when grain yield potential is lower than in floral buds and flowers with no frost exposure. This inference is reinforced by the number of grains originating from frost-exposed floral buds and flowers, which generated siliques with a number of grains similar to non-exposed floral buds (Table 7). The non-aborted siliques of cold-stressed plants

presented practically a null number of grains (Tables 6 and 7), and the level of frost damage to floral buds and flowers was lower than in siliques. Grain dry matter production in frost-exposed plants was therefore probably derived from those reproductive structures in which ovule fertilization occurred after frost exposure (floral buds and flowers). Pollen grains can be damaged by cold stress but, due to canola's high pollen production, a pollen grain viability of 20 % is sufficient for ovule fertilization without compromising grain yield (Lardon & Triboi-Blondel, 1995).

These insights into the responses of the canola to frost will enable a better understanding of frost damage to field crops in the environment of Southern Brazil, given its high climatic variability during the crop reproduction cycle. Our study results also assist in identifying and quantifying frost damage, in order to establish risk levels associated with grain yield loss in canola.

Conclusions

Our results enabled us to draw the following conclusions for canola plants in their reproductive phase. 1. Frost simulation (air temperature of –6°C) in a controlled environment causes death in non-acclimated canola plants. 2. Frost reduces the total dry matter, number of reproductive structures, grain dry matter, number of siliques per plant, number of grains per silique, and number of viable ovules of canola plants. 3. Canola plants show ability to increase tolerance to frost damage when exposed for three days at air temperature with acclimation potential, except for damage to ovules in the ovaries.

Additionally, we found that frost exposure causes abortion of siliques of different developmental sizes, even those over 4 cm in length. However, cold acclimation is not efficient in reducing frost-related ovule abortion in canola, irrespective of ovule developmental stage.

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Contributions of authors

Conceptualization: Kovaleski, S., Heldwein, A.B., Dalmago, G.A. Data acquisition: Kovaleski, S., Heldwein, A.B., Dalmago, G.A., Gouvêa, J.A. de. Data analysis: Kovaleski, S., Heldwein, A.B., Dalmago, G.A., Gouvêa, J.A. de. Design of Methodology: Kovaleski, S., Heldwein, A.B., Dalmago, G.A., Gouvêa, J.A. de. Writing and editing: Kovaleski, S., Heldwein, A.B., Dalmago, G.A., Gouvêa, J.A. de.

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Danos causados pela geada durante a fase reprodutiva da canola (*Brassica napus* L.), em ambiente controlado

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INFORMAÇÕES

RESUMO

Este estudo avaliou os danos causados pela geada e os benefícios da aclimatação ao frio durante a fase reprodutiva da canola. As plantas foram expostas a aclimatação ao frio e simulação de geada no início da floração, durante a floração plena e no início do enchimento dos grãos. Foram avaliados: mortalidade de plantas, número de óvulos viáveis e síliquas abortadas, botões florais e flores, número de grãos por síliqua, matéria seca total das plantas, número de síliquas, total de síliquas abortadas e matéria seca de grãos. A geada causou a morte de plantas não aclimatadas e aumentou o abortamento de síliquas, botões florais e flores, reduzindo assim o número de síliquas e matéria seca dos grãos. A geada impediu, principalmente, o desenvolvimento de embriões nas síliquas, enquanto óvulos viáveis foram encontrados nos botões florais e nas flores. Durante a fase reprodutiva, as plantas de canola têm capacidade limitada de aumentar sua tolerância a danos causados pela geada. No entanto, as síliquas originada dos botões florais e das flores abertas continham um número maior de grãos do que as síliquas após a exposição à geada. Portanto, desde o início da floração até o início do enchimento dos grãos, os botões florais e as flores são responsáveis por produzir a maior parte da matéria seca dos grãos quando há ocorrência de geada.

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