Final Report. March, 2013

SCREENING CANOLA FOR "FLOWER BLASTING" TOLERANCE

CARP Project 2012-20

The following information should be treated as confidential between AAFC and CARP until verification.

March 25, 2013

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Summary:

In the field, air temperatures greater than 30°C causes reduced fertility, smaller seed size and lower yield in canola. Future climate change scenarios predict warmer temperatures for the major canola growing regions of Canada. The objective of the research was to develop a growth cabinet screening protocol to select for heat stress tolerant canola cultivars. The protocol was used to many canola cultivars to determine if they had greater heat tolerance than Westar. In the initial experiments to develop a protocol we found that growth cabinet temperatures greater than 27°C (29 and 30°C) resulted in complete raceme sterility in the conventional cultivar Westar and the test hybrid Invigor. The test was not designed to test for extremes in temperature response but to determine if new lines were better than older ones in tolerating heat. A test protocol was developed where cultivars were grown to flowering in a control cabinet set at 15/20°C (night/day). At flowering half of the plants were transferred to a warm cabinet (22/27°C) while the other half remained in the control cabinet. The percent raceme fertility was calculated by counting the number of pods that had at least one viable seed and dividing it from the total number of pods produced in two weeks of flowering in the warm cabinet. We also looked at the total number of seeds produced in that two week period. A heat tolerant cultivar had higher percent fertility in the warm cabinet than Westar and produced nearly as much seed in the warm cabinet as in the cool growth cabinet. Of the 47 cultivars screened, 14 had lower heat stress fertility than Westar, meaning that they produced fewer fertile pods (-63 to -10 percent fertility) when heat stressed, than the check. Eighteen of the cultivars had similar heat stress tolerance as Westar (-9 to 9 percent fertility) and 15 had greater tolerance than Westar (>10 percent fertility). Pollen germination experiments in artificial media found that as the temperature of the media increased past 33°C the number of germinating pollen grains producing healthy pollen tubes decreased. At 27°C, which was the heat stress temperature, all of the cultivars produced healthy germinating pollen indicating that pollen germination was not a factor affecting heat stress tolerance.

There is variability for heat stress tolerance among current canola cultivars. Many of the canola breeding companies have been inadvertently breeding for heat stress tolerance by conducting

their initial breeding in high heat environments. Higher heat stress temperatures than 27°C need to be tested in future experiments.

Project Objectives: To examine current canola cultivars for flower blasting (heat stress) tolerance and provide the information to growers and breeders across Canada. To develop a simple test for flower blasting that can be adopted by breeders. Pollen germination *in vitro* was tested at several temperatures to examine the influence of heat stress on pollen germination and vigour. Full details of the heat stress and pollen germination protocol are in the appendix.

Heat Stress Growth Protocol:

The goal of the study was to examine the fertility of canola plants exposed to heat stress during a 2 week period of flowering compared to plants grown in cool conditions. New cultivars were compared to a control; Westar.

Plastic pots, 15cm in diameter and 18 cm tall, were filled with 2.2 kg of a 2:1 soil:sand mixture. The pots were watered on an automated system that delivered the same amount of per pot. The system was adjusted on a weekly basis to maintain well watered conditions and avoid moisture stress. Plants were fertilized with a 20:20:20 solution plus magnesium sulfate once per week after the third leaf. In each run, 7 to 10 canola cultivars (9 lines and 1 check, Westar), were replicated 4 times for a total of 40 plants. The plants were grown in a cool (20/15 °C, day/night, 17h) walk-in controlled environment cabinet until the onset of bolting (Harper-Berkenkamp 1975; stage 3.2). Two plants from each cultivar were transferred to a warm (22 to 27°C) growth cabinet while the other 2 plants remained in the cool cabinet for the duration of the experiment. In the warm cabinet, temperature was increased by 1°C per hour from 22 to 27°C, where it was maintained for 7 hours before being decreased by 1°C per hour to 22°C. Initial experiments with temperatures greater than 27°C resulted in complete raceme sterility and the objective was to find lines with heat stress better than Westar not to create complete raceme sterility. During flowering, the racemes were manipulated manually each day to increase the transfer of pollen from anther to stigma. A tag was placed at the first flower and another at the flower on the raceme two weeks after the beginning of flowering in each growth cabinet. Plants from the warm chamber were transferred back to the cool chamber after two weeks of heat stress where they remained until maturity. Lateral racemes were removed as they appeared. At maturity, the number and type of pods were recorded on the raceme for the two week period during flowering.
A fertile pod was any pod that produced at least one viable seed. There were three types of sterile pods: 1) a pod that had aborted and fallen off the raceme, leaving a scar or just a pedicle; 2) a parthenocarpic pod which had partially formed but contained no seed; 3) a long and thin pod that contained no seed. The number of seeds per pod was counted and recorded and the percent fertility and percent seed number calculated as follows:

Percent Fertility: the number of fertile pods per raceme (containing at least one seed) divided by the total number of pods per 2 week period. The difference between the warm cabinet cultivar percent fertility and the Westar warm cabinet percent fertility represented the amount the cultivar differed from the check -- or how much better the plant was at producing a fertile pod when grown at warm temperatures compared to Westar.

Percent Seed Number: the number of seeds produced during a two week period, per raceme, divided by the number of seeds produced on Westar for the same duration and cabinet temperature. This was calculated for both the cool and warm cabinets.

Pollen Germination Protocol:

Pollen sterility is often identified as the source of low yield when the plants are subjected to high temperatures. The purpose of this part of the experiment was to study canola pollen germination *in vitro* as affected by cultivar, pre-germination plant temperature and pollen germination temperature. Pollen germination was studied across a temperature gradient (20 to 40°C) established using a PCR thermocycler. The viability (germination) and vigor (1 to 5 rating) of *B. napus* pollen from cool and warm grown plants was compared. Cultivar differences were examined.

Germination media

A liquid *in vitro* germination media for Brassica contained 500 µl of 100 mM magnesium sulfate ($MgSO_4$), 500 µl of 500 mM potassium chloride (KCl), 500µl of 500 mM calcium chloride ($CaCl_2$), 500 µl of 1.0% H_3BO_3, 5 g of sucrose, 10 g of PEG 8000, and enough nano pure H_2O for the media to reach a total volume of 50 mL. This resulted in a germination media containing the concentrations in table 1.

Table 1: Final concentrations of solutions in the germination media

Solution	Final Concentration
$MgSO_4$	1 mM
KCl	5 mM
$CaCl_2$	5 mM
H_3BO_3	0.01%
Sucrose	10%
PEG 8000	20% w/v

0.1M NaOH was also added to adjust the pH to 7.5

Collecting pollen samples

Pollen was collected from plants grown in the warm and cool cabinets using an aspirator made by connecting an electric vacuum pump through a Büchner flask, which had a control valve on one side connected to a piece of tygon tubing. A plastic disposable pipette tip on the end of the tube, was used to collect the pollen onto a piece of nitex mesh fabric. This allowed the pollen to be caught by the nitex fabric when being sucked off the anthers of 1-2 day old canola flowers. The pollen was then scraped off the nitex into a small centrifuge tube. This was done for each plant sampled.

The tubes were briefly centrifuged to bring all the pollen to the bottom. A pollen suspension was made using 280 µl of germination media added to the tube, which was tapped and shaken to equally distributed the pollen in the suspension. Twenty µl of the pollen suspension, from

each treatment, was gently placed on top of 150µl of the germination media in a well of a 96-well (12 columns x 8 rows) PCR plate. The tube was tapped after each sub-sample was removed from it and deposited into a well. Each cultivar x heat treatment occupied one row of the plate in the thermocycler; therefore, the pollen was germinated at 12 different temperatures. Once the suspension was added to each well, the PCR plate row was covered with masking tape, and a damp cotton cloth the size of the PCR plate was placed on top. The thermocycler was run for 8 hours with the temperature set to 30ºC with a gradient of +/- 10 ºC, creating a temperature range from 20 to 40 ºC (Table 2). After the 8 hour cycle was completed the PCR plate temperature was reduced to 4ºC to terminate pollen germination before it was examined under the microscope.

Table 2: Temperatures of each well on the 96-well PCR plate

Well	Temperature (ºC)	Well	Temperature (ºC)
1	19.9	7	30.6
2	20.1	8	33.4
3	21.1	9	36.0
4	22.8	10	38.2
5	25.1	11	39.9
6	27.8	12	40.9

Analyzing pollen germination in the wells

A picture was taken from each well using a Zeiss Axioplan 2 microscope (5X objective, bright field). The “Z-stacks” feature of the microscope software was used to create an image with multiple layers, or focal distances, to overcome the curved nature of the surface of the germination media in the well so that more pollen could be in focus in each image. For each image, a circle with a radius of ~671 µm was traced around the area of pollen to be analyzed. The total number of pollen grains per area was counted as well as the number of germinated pollen grains to determine the percent pollen germination; ((number of germinated pollen grains / total number of pollen grains) x 100). Each picture of the pollen in a well was scored to determine whether pollen germination was affected by temperature. Only pollen found completely within the traced circle was analyzed. One pollen score was made per picture. To score the quality of pollen tubes, a scale of 1-5 was used (Figure 1). The percent germination accounted for pollen viability, and the visual rating account for the vigour of the germinated pollen tubes. These two numbers were multiplied to produce a number to measure of the overall success of the pollen grains and represent **pollen vitality** with a larger number being better than a smaller one.

A general linear model was used to analyse the statistical differences in pollen vitality among cultivars as influenced by pre-germination cabinet temperature (heat stress of the plants) and the pollen germination well temperature (heat stress of the pollen). The test was run twice in time and run was used as replication.

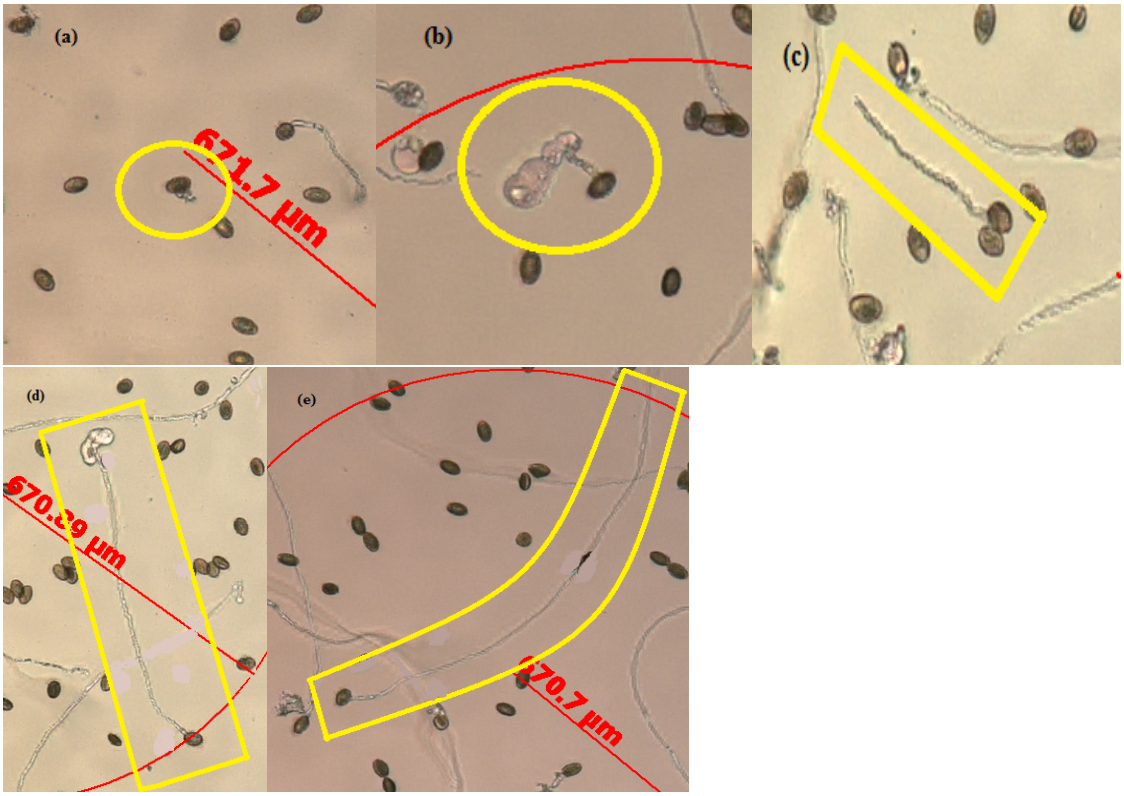

Figure 1: Pollen germination ranking (1-5); (a) 1 = a ruptured pollen grain or no pollen tube present; (b) 2 = short, ruptured pollen tubes; (c) 3 = short intact pollen tubes; (d) 4 = long, ruptured pollen tubes; (e) 5 = long, healthy pollen tubes. Images taken with the Zeiss Axioplan 2 microscope.

Progress and Research Findings April 01, 2010 to March 31, 2013.

- Grant approved April 01, 2010.
- Contract between Canola Council and AAFC signed November 8, 2010.
- AAFC Financial Coding for project established November 19, 2010.
- Seed from more than 50 different canola cultivars was collected from Canada and Australia.
- Two Research Transfer Agreements were signed: U of Manitoba for 4 cultivars and Pioneer for 20 coded lines.

Heat Stress Testing

- Controlled environment facilities were used to test canola cultivars for heat stress tolerance.
- A heat stress protocol was developed. See methods and Appendix.
- Seven Coop students were hired beginning from January 10, 2011 to March 31, 2013. The majority of the funds were spent on students.
- All growth stages from the beginning of bolting (HB 3.1) through to the end of flowering (HB4.3) are susceptible to heat stress temperatures.
- A daytime high of 29 ºC in the growth cabinet resulted in abnormal floral production and complete flower sterility in the test cultivars.

- The heat stress temperature was reduced from 29 to 27ºC in the growth cabinets and the temperature ramped up from 22 ºC in the morning (sunrise) to a mid-day high of 27 ºC and then ramped down to an overnight temperature of 22ºC.
- The objective of the project was to find cultivars with improved heat stress tolerance compared to Westar not to show that all canola cultivars were susceptible to excessive heat stress.
- We have found that buds maturing into flowers (HB 3.2 to 4.1) are most susceptible to heat stress. Flowers that develop to bud in the cold cabinet prior to being transferred to the hot cabinet have some fertility and usually produce seed, but if the flowers develop and open in the warm cabinet they have reduced fertility. The most fertile pods in a heat stressed plant occur at the bottom of the raceme.
- Figure 2 illustrates a fertile plant, a plant with the majority of fertile pods on the bottom of the raceme and two sterile plants. When a flower is pollenated but the seeds not successfully fertilized the pod will develop to a certain extent and stop. We called these chubby little pods, parthenocarpic.
- A total of 16 screening trials were conducted in controlled environment facilities. Three of these were used to establish the heat stress protocol. Four had to be abandoned and repeated due to equipment failure or insect damage. Nine trials were successfully completed and heat stress was examined in 47 cultivars.
- The cultivar Westar was used as a check in every trial and all cultivar were compared to it.

Figure 2. Four types of plants obtained in the growth cabinet experiments. Plant A was grown in the cool growth cabinet (20/15ºC) and is fertile with well developed pods; plant B was grown in the warm (27/22) growth cabinet and is completely sterile, no seed production, and has short seedless pods. Plant C, which was grown until bud elongation in the cool cabinet and transferred to the warm cabinet, has the first 19 pods fertile and the rest sterile. Plant D was grown in the warm cabinet and shows the production of short, fat, seedless pods called parthenocarpic pods.

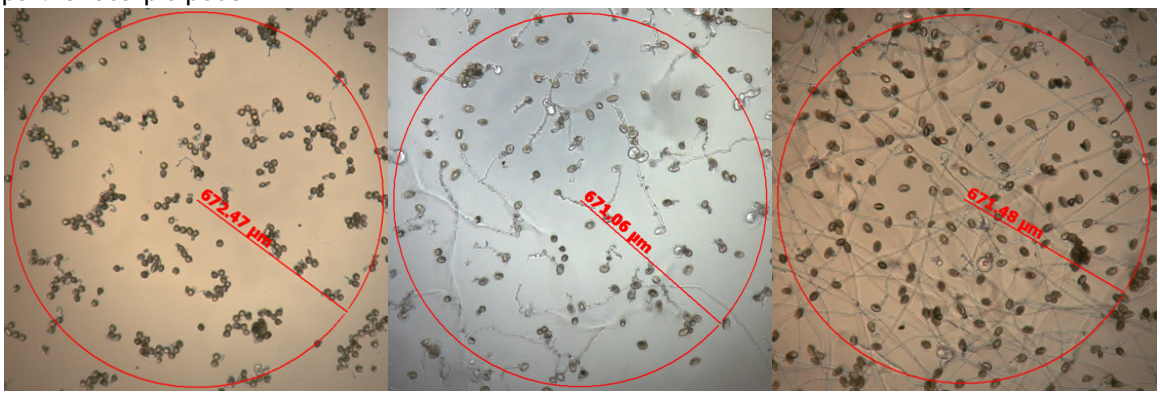

Fig. 3. From left to right; low germination, low vigour (40.9 ºC); medium germination, medium vigour (33.4 ºC); high germination, high vigour (21.1 ºC) (Zeiss Axioplan 2)

- Heat stress tolerance was measured by two methods. The **Percent Fertility** was the number of fertile pods, on the main raceme, produced in two weeks of exposure to heat stress, divided by the total number of pods produced in two weeks. The percent fertility relative to Westar was the difference between the cultivar and Westar percent fertility in

the warm cabinet. The second method to examine heat tolerance was the **Percent seed number** produced during two weeks of heat stress compared to the number of seeds produced on Westar.

- Of the 47 cultivars tested to date, 15 had greater percent raceme fertility in heat stress than Westar (>10% raceme fertility), 18 were similar (-10 to 10% raceme fertility) and 14 of them had lower raceme fertility (<-10% raceme fertility). There was almost a normal distribution of response (Table 3).
- The most heat stress tolerant lines had high raceme fertility in the warm cabinet relative to Westar, almost equal number of seeds in cool and warm cabinets, and had a higher percentage of seeds in the warm cabinet compared to Westar. Of the 15 cultivars that had greater raceme fertility than Westar there were 7 cultivars that had a small difference in seed number between cool and warm chambers and much higher level of seed production in the warm cabinet than Westar. The best performing cultivars were 11PH0078, DK72-65, 11PH0090, 11PH0088, 11PH0091, 11PH0081, 11PH0080.
- Only 2 cultivars from Australia showed higher heat stress fertility than Westar and these were likely bred in Canada and just distributed in Australia. Australian canola is seeded in the fall; grows slowly over winter and flowers in mid spring when the temperatures are still relatively cool. Heat stress tolerance may not have been a trait of selection in Australia; especially for cultivars bred there.
- While seven of the 15 cultivars produce a similar number of seeds in both warm and cool chambers for the most part canola produced more seeds in the cool cabinet, even though there were fewer pods produced during the two week exposure period.
- Eleven of the coded lines from Pioneer had significantly greater heat stress tolerance than Westar while 9 did worse than the check. It is assumed that these are new potential cultivars so far show great promise. These lines were selected in southern Ontario (Georgetown) where heat stress during reproduction would be routinely higher. Breeding companies should be selecting future cultivars in warmer environments than they are expected to grow in to incorporate more heat stress tolerance in their future cultivars.

Pollen Germination:

- Pollen germination, as influenced by cultivar, growing temperature and germination temperature was tested using a PCR thermo-cycler with a gradient established from 20 to 40 ^{o}C across 12 wells (see protocol in appendix and methods).
- Heat stress affected the number of pollen grains germinating and the vigor of the pollen tubes. Figure 3 shows the difference between pollen grains that had germinated poorly with poor vigour (classified as a 1), had medium germination and vigour (classified as a 3) and a vigorous pollen germination and growth (classified as a 5).
- **Pollen Vitality** was the product of percent pollen germination and a pollen vigour rating of 1 to 5.
- An analysis of variance done on pollen vitality revealed that there were significant differences among cultivars, pre-germination heat stress and germination temperature. Table 4.
- The environment where the pollen was produced influences its vitality. Five of the seven cultivars had higher vitality when the plants were grown in the cool chamber and two seemed to have higher vitality at high temperatures when the plants were grown in the warm chamber. Table 5.
- There were differences among cultivars for mean pollen vitality ranging from 18 to 84 in the warm chamber and 35 to 76 in the cool chamber.

Table 3. Heat stress test results for canola cultivars relative (rel) to Westar (WES).

Cultivar	Company	Cool pod num.	Warm pod num.	Percent Fertility rel. to WES	Seed # diff. (cool - warm)	Percent Seed # rel to WES
HYOLA 676 CL	Pacific Seeds, Australia	61	50	-63	56	-99
HYOLA 571 CL	Pacific Seeds, Australia	66	72	-37	736	-71
Pioneer 06N7851	Pioneer, Australia	52	51	-35	-85	45
CB TANAMI	Canola Breeders Australia	39	42	-30	439	-61
CB ARGYLE	Canola Breeders Australia	49	59	-24	142	-60
11PH0082	Pioneer, Canada	54	51	-22	109	-63
11PH0079	Pioneer, Canada	28	44	-19	144	-86
11PH0087	Pioneer, Canada	53	70	-16	725	7
Pinnacle	Unknown, Australia	43	18	-15	383	-80
11PH0085	Pioneer, Canada	59	71	-14	1103	10
Pioneer 43C80	Pioneer, Austalia	23	31	-14	-22	-75
SURPASS	Pacific Seeds, Australia	74	58	-13	710	-18
11PH0084	Pioneer, Canada	58	59	-13	844	-2
AC5-C7	Unknown, Canada	98	106	-10	1	-92
NX4-104 RR	Dow Agro Science, Canada	66	63	-8	40	-55
CB JARDEE	Canola Breeders, Australia	36	46	-8	206	-19
CB SCADDAN	Canola Breeders, Australia	44	58	-8	24	-18
G31046	Unknown, Canada	59	59	-7	292	-26
STELLAR	U. of Manitoba, Canada	63	76	-4	700	-15
Test 10-19	Unknown, Canada	65	85	-4	196	-11
11PH0083	Pioneer, Canada	56	65	-4	515	-12
11PH0073	Pioneer, Canada	27	65	-4	-96	24
71-45 RR	Monsanto, Canada	73	89	-4	279	-8
NX4-105 RR	Dow Agro Science, Canada	61	63	-3	220	-56
NX4-102	Dow Agro Science, Canada	65	72	-1	84	-47
NX4-101	Dow Agro Science, Canada	65	79	-1	333	-13
CB Junnee	Canola Breeders, Australia	58	62	-1	465	-16
KAROO	Canola Breeders, Australia	50	44	1	428	-37
11PH0092	Pioneer, Canada	70	77	3	987	40
11PH0074	Pioneer, Canada	65	86	4	309	167
3465 RR	Monsanto, Canada	60	66	6	671	49
11PH0089	Pioneer, Canada	63	91	6	201	222
Pioneer 44C79	Pioneer, Australia	15	52	21	-370	138
11PH0086	Pioneer, Canada	60	72	22	523	470
71-25 RR	Monsanto, Canada	65	75	23	241	103
HYOLA 50	Pacific Seeds, Australia	63	69	23	477	55
11PH0078	Pioneer, Canada	60	68	24	-132	240
DK72-65	DeKalb, Canada	60	71	24	82	284
11PH0090	Pioneer, Canada	62	75	30	286	268
11PH0088	Pioneer, Canada	42	62	33	-37	525
APOLLO	U. of Manitoba, Canada	62	62	36	524	164
11PH0091	Pioneer, Canada	69	89	40	456	438
11PH0081	Pioneer, Canada	54	62	42	397	320
11PH0077	Pioneer, Canada	69	87	42	648	380
11PH0080	Pioneer, Canada	54	70	43	133	408
11PH0076	Pioneer, Canada	50	60	49	84	114
11PH0075	Pioneer, Canada	40	39	55	-97	19

- The average pollen germination is low with only around 20% of the pollen grains germinating (data not shown). Pollen germination is greatest at well temperatures between 27 and 30°C but the pollen vigour, a measured by pollen tube length and health is best at low temperatures (20 to 25°C). It is unknown how artificial pollen germination reflects actual germination on the stigma.
- Preliminary conclusions are that pollen is viable up to 33.4 °C (Figure 5). Heat stress does affect pollen germination but in the temperature range experienced in the growth chamber and in the field in western Canada it is not likely that a reduction in the ability of the pollen to germinate is the cause of heat stress induced flower blasting. That being said, pollen germination tests are done in a high humidity environment, removed from humidity and drought stresses experienced on the stigma surface. These conditions are very difficult to simulate and therefore, may be impossible to use as screening tools for heat stress tolerance.
- Preliminary results have shown that temperatures between 23ºC and 35 ºC resulted in optimum germination for *Brassica napus* pollen. At temperatures lower than 23 ºC there was lower germination but those that did germinate were healthy. While there were several pollen grains germinating from 32 to 35 ºC they were not as healthy as those at lower temperatures. It appears that between 26 to 30ºC is the optimum temperature for pollen germination and this would rule out pollen viability as the cause of heat stress reduced fertility in canola.

Table 4: Combined GLM analysis of variance for pollen vitality of cultivars from hot and cold cabinets.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Run	1	2445.8	2445.8	2.6	0.1116
Cabinet	1	10074.9	10074.9	10.5	0.0014
Cult[1]	6	74002.9	12333.8	12.9	<0.0001
Well Temp[2]	11	246098.0	22372.5	23.4	<0.0001
Cult*Temp	66	100567.2	1523.8	1.6	0.0091
Cabinet*Cult	6	17186.1	2864.4	3.0	0.0083
Cabinet*Temp	11	16600.5	1509.1	1.6	0.1090
Cabinet*Cult*Temp	66	69141.1	1047.6	1.1	0.3163

[1] Cult refers to cultivar
[2] refers to the temperature of the well

Table 5: Vitality of germinated pollen at twelve well temperatures for seven cultivars grown in hot and cold cabinets

	Temperature (°C)												
Cult[1]	**19.9**	**20.1**	**21.1**	**22.8**	**25.1**	**27.8**	**30.6**	**33.4**	**36.0**	**38.2**	**39.9**	**40.9**	**Mean**
	Hot (22 to 27 °C)												
PH91	74.2	71.5	73.7	68.6	84.9	58.0	50.9	47.1	17.1	11.1	9.0	7.9	47.8
PH80	36.5	52.0	17.2	64.0	69.6	74.0	62.5	90.2	40.7	3.5	3.5	9.4	43.6
PH81	63.1	58.8	15.8	97.3	121.4	122.3	141.1	48.1	3.4	2.8	8.2	31.6	59.5
HYO	57.6	60.3	44.0	42.8	61.6	41.5	37.8	44.8	50.1	12.5	10.0	11.8	39.6
71-25	135.7	110.3	68.1	86.3	76.6	86.8	144.6	118.3	45.8	37.0	24.1	75.0	84.1
APO	79.9	60.7	37.3	40.7	42.7	50.8	58.7	18.2	8.0	9.7	7.0	16.4	35.8
STE	2.8	26.3	16.2	26.7	27.3	33.6	30.6	16.7	3.2	15.9	7.3	19.0	18.8
LSD	48.2	104.1	55.5	40.9	35.2	74.6	91.1	61.2	68.9	29.0	38.7	103.3	16.1
	Cold (15 to 20 °C)												
PH91	55.1	52.6	53.2	61.6	51.3	32.5	88.8	49.9	34.3	14.3	24.4	5.0	43.6
PH80	125.4	64.8	84.7	91.6	131.4	105.0	79.6	43.3	24.1	9.7	19.8	23.8	66.9
PH81	113.6	84.0	113.1	152.5	69.8	123.8	118.4	70.2	17.8	26.0	8.3	19.5	76.4
HYO	114.0	97.8	89.0	94.2	67.3	30.6	64.9	129.2	54.5	0.0	5.9	9.4	63.1
71-25	62.5	137.3	10.3	169.4	115.0	81.8	80.8	125.4	8.8	0.0	9.2	4.6	67.1
APO	115.8	126.6	63.5	37.4	95.8	86.9	85.2	13.6	8.1	0.0	0.0	11.7	53.7
STE	71.1	57.3	33.3	64.8	37.8	39.3	36.7	19.5	11.7	16.9	11.1	20.4	35.0
LSD	90.9	71.1	63.2	135.4	148.3	67.7	127.6	42.5	50.8	24.4	28.4	25.9	19.4

[1] Cult refers to cultivar

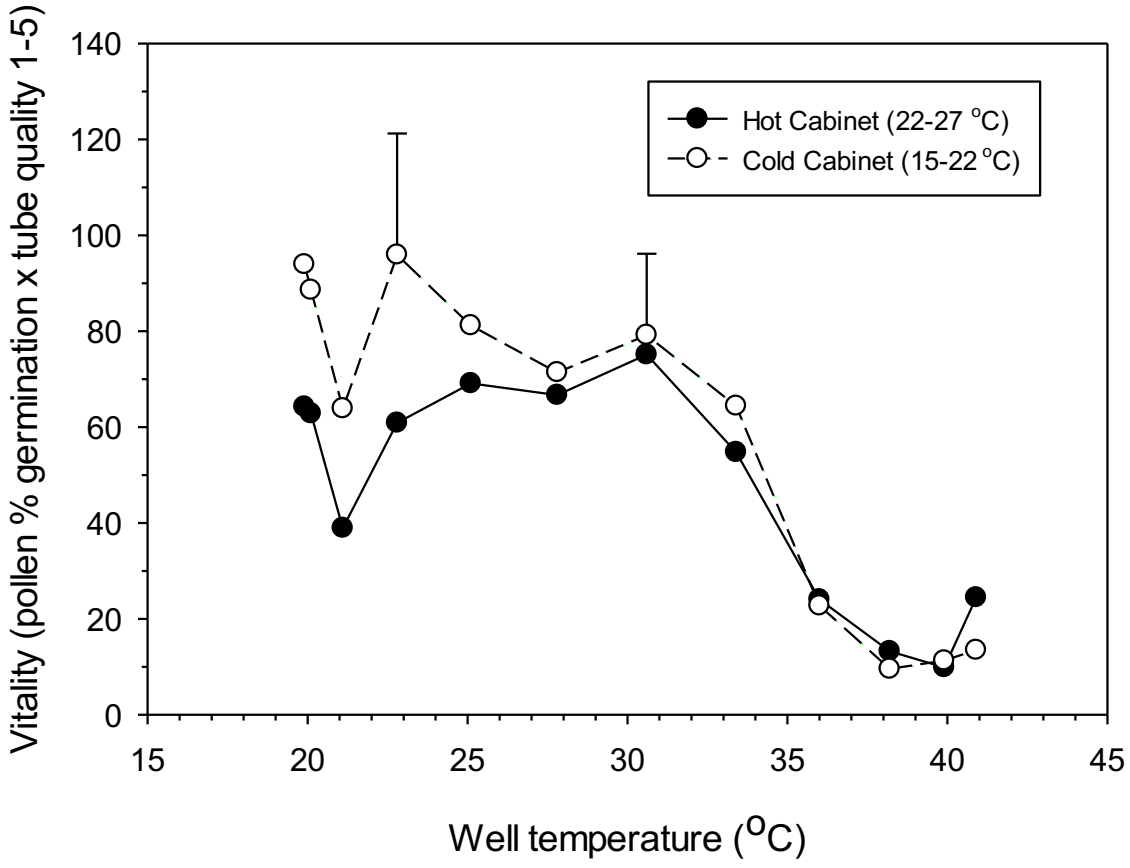

Figure 4. Average pollen vitality (germination x vigour rating) across a temperature gradient ranging from 20 to 40°C

Conclusions:

Several of the canola cultivars examined demonstrated greater fertility than Westar in the warm environment and a less than 10% difference in seed number between racemes grown in the warm and cool environments. These cultivars can be considered to be more tolerant to heat stress during flowering than others. Our results show that cultivars can be tested in controlled environments for heat stress rather simply. Many of the cultivars tested that exhibited heat stress tolerance are new lines developed in the Pioneer breeding program. It is not known if they are current or future cultivars because they are coded, but this does indicate that selecting for yield in a hot environment, such as southern Ontario, may be an effective way of selecting for heat stress. Many of the older roundup ready cultivars for example may not have had the benefit of being selected in warmer years.

Future heat stress tests will concentrate on the cultivars that have already been found to be more heat stress tolerant. The results of this experiment are encouraging because they show that new cultivars of canola are being developed with greater heat stress tolerance than the older ones. This will be a required feature of high yielding cultivars in the future.

Budget Allocation

CARP costs of 3 years of Research Funding @ 72,000 per year

Item	Allocation
AAFC Overhead	10,800
Student Salary (4x 10,000)	57,600
Growth Cabinet and minor equipment	3,600
Total	72,000

Appendix

Heat Stress Protocol Growth Cabinet:

The following is the methodology used to determine the influence of warm temperatures on the number of fertile pods and seeds produced per raceme.

- Four canola plants from each cultivar were grown in 15 cm diameter plastic pots in 2:1 soil:sand mixture under well watered conditions. Several seeds of each cultivar were planted in a pot and thinned at the first leaf to one plant per pot.
- Soil moisture was measured regularly and kept between 17 to 22% moisture by volume using an auto watering system. The saturation water capacity of the soil in a pot was 26% moisture by volume.
- Plants were well fertilized with 100 mls of ½ strength 20:20:20 supplied every 2 weeks.
- Two growth cabinets were used for the experiment: cool (20/15^{o}C, day/night, 17 h daylength) and hot (27/22^{o}C, day/night, 17 h daylength).
- The temperature in the hot cabinet was ramped up at 1^{o}C per hour from 22 to 27, remained at 27 for 7 hours and was ramped down to 22 overnight.
- The plants were grown in the cold cabinet until first bud visible and bolting initiated. This corresponds to the Harper Berkenkamp (1975) 3.2 growth stage.
- Two of the four plants from each cultivar were transferred to the hot cabinet at HB 3.2.
- When the first flower opened on plants in either cabinet a tag containing the date was placed below the flower.
- Fourteen days after first flower the plants from the hot cabinet were transferred back to the cool cabinet and a tag placed above the last opened flower in the two week period. The plants remained in the cool cabinet until the seeds were fully formed HB 5.3.
- During flowering the flowers were pollinated with pollen from their own anthers to ensure that asynchrony of stigma and anther development was not the cause of sterility. This was done by manipulating each raceme with a gloved hand, wiping the pollen from the raceme between plants.
- During ripening the lateral racemes that developed were pruned from the plants and watering reduced gradually as the lower main raceme leaves senesced.
- Three to four weeks after the plants were transferred back to the cool cabinet the pods on the main raceme were rated for fertility. Each main raceme was examined for the number of fertile pods, and the number of seeds per pod.
- Hot transfer plants were compared to plants that remained in the cold cabinet to determine the Percent raceme fertility.
- Percent heat stress was compared to Westar to determine the cultivars relative performance.

Pollen Germination Protocol

Pollen viability from the hot and cold cabinet was measured to determine if heat stress results in a general reduction in germination rate. A thermal gradient from 20 to 40 C was used with pollen collected from 5 or 6 flowers at one time.

- The liquid *in vitro* germination media for *B. napus* pollen contained 500 µl of 100 mM magnesium sulfate ($MgSO_4$), 500 µl of 500 mM potassium chloride (KCl), 500µl of 500 mM calcium chloride ($CaCl_2$), 500 µl of 1.0% H_3BO_3, 5 g of sucrose, 10 g of PEG 8000, and enough nano pure H_2O for the media to reach a total volume of 50 mL., 0.1M NaOH was also added to adjust the pH to 7.5.
- Pollen was collected from 1 to 2 day old anthers from 5 to 6 flowers per plant grown in the hot and cold cabinets, using an aspirating technique where suction was used to remove the pollen from the anthers and collect it on a nitex membrane. The pollen was then transferred off the nitex into a small centrifuge tube.
- After centrifugation to bring the pollen to the bottom of the tube a suspension was made using 280 µl of germination media was added to the tube, and then the tube was shaken to create an equally distributed pollen suspension.
- A 96 well (8 rows of 12 columns) PCR plate was used for pollen germination. The 12 columns in a row were filled with 150 µl of the germination media and then 20 µl of the pollen suspension was added onto the surface of the germination media for each well.
- Once the suspension was added to each well, the PCR plate was covered with masking tape, and a damp cotton cloth the size of the PCR plate was placed on top to reduce the amount of evaporation from each well
- The 96 well PCR plate was put into a PCR Thermocycler for 8 hours with the temperature set to 30ºC with a gradient of +/- 10ºC. This created a temperature gradient from 20 to 40 ºC across the row. After 8 hours the thermocycler was programed to hold the pollen in status by chilling them to 4ºC. Wells 1-12 had the following temperatures throughout the cycle:

Well	Temp ºC	Well	Temp ºC
1	19.9	7	30.6
2	20.1	8	33.4
3	21.1	9	36.0
4	22.8	10	38.2
5	25.1	11	39.9
6	27.8	12	40.9

- After 8 hours of germination the PCR plate was examined on a microscope, pictures of each well taken and the pollen grain in the pictures counted and scored for viability on a 1 to 5 scale with 1 representing perfect germination and 5 no germination.