FINAL PROJECT REPORT
Canola Agronomic Research Program (CARP)

The Final Project Report should fully describe the work completed for the project and note the personnel involved. It should also note any deviations from the original plan and any corrective steps that were taken during the course of the project. A complete statement of expenses should be included.

Project Title: Developing a rapid method to evaluate pod-drop in canola

Research Team Information

Lead Researchers:		
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Research Team Members		
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Project Start Date: May 2013 **Project Completion Date:** April 2016

CARP Project Number: 2013-13

Instructions: This Final Project Report shall be completed and submitted on or about March 31st of the project completion year. The Lead Researcher of the project in question shall complete and submit the report on behalf of his/her complete research team.

This Report is a means by which to provide a final summary on the status and activities of the project. Details may be general in nature unless major issues or changes arise (e.g., change of scientists, significant change or delay of activities) including impacts on budgets. Please note that financial reports of major impact on budgets.

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1. Forecasted Date of Completion:

Completed.

2. Status of Activity: (please check one)

_____ Ahead of Schedule _____ On Schedule _____Behind Schedule X Completed

Comment:

3. Completed actions, deliverables and results; any major issues or variance between planned and actual activities.

Background

Canola is a crop associated with large seed losses before and at harvest (Gulden et al. 2003, Gan et al. 2008, Cavalieri et al. 2016). Two phenomena contribute to these seed losses, i) pod shatter where siliques open and lose their seeds while still attached to the plant and ii) pod drop, where entire siliques break at the petiole and drop to the ground. In a recent study, pod drop accounted for about 35% (± 16%) of total yield loss among a series of canola genotypes (Cavalieri et al. 2014) and Gan et al. (2008) also showed different pod drop potential among Brassica species, however, the differences among the species were only observed under high shatter conditions. Pod drop is less well understood than pod shatter and also is more difficult to measure. To assist with understanding pod drop we initiated the use of force gauges to measure pod-retention resistance, but have not determined the variance components or a clear relationship between this measurement and actual pod drop. To further develop this method, the following objectives were addressed in this research:

1) Refine the number of measurements and rachis type and position from where to obtain meaningful pod-retention resistance measurements.

2) Use this method to determine and validate a relationship between pod-retention resistance and pod-drop across a number of genotypes and environments.

Completed actions, Materials & Methods and Results

All the field activities planned in the project proposal were completed. For objective 1, field experiments (method refinement experiments) were established either in 2013 or 2014 at two different locations, Carman, MB and Saskatoon, SK. During the first field season (2013), the experiment at Carman was seeded twice on two different dates (23rd May and 7th June), while only one seeding date was used in Saskatoon (19th May). The early planted experiment at Carman was lost in 2013 due to untimely hail on the 30th of August. During the second field season (2014), the experiment was seeded twice at Carman on two different dates (15th and 30th May), while only one seeding date was used in Saskatoon (14th May). The early planted experiment at Carman was only used for the pod retention resistance measurement; the high frequency in precipitation during the first months of the field season caused a sclerotinia outbreak which compromised the final harvest and pod drop collection. For the objective 2, Canola Performance Trials (CPT experiments) from the 'Co-operative Tests' were used to take measurements with the pod-retention-resistance method either in 2014 or 2015. In 2014, the two locations designed were Carman, MB and Outlook, SK. In 2015, a larger number of locations were used either in Manitoba (trials located in Thornhill (DL Seeds) and Elm Creek (Cargill)), or in Saskatchewan (trials located in Saskatoon (DL Seeds), Wakaw (ICMS), and Melfort (Bayer)). Seed loss samples were collected with catch trays only in 2015, after having obtained the required authorization from the cooperators.

Crop management

Method refinement experiments (Obj. 1)

Six canola varieties were used in this study in 2013 and 2014 (four glyphosate- and two glufosinate-resistant varieties - Dekalb 73-15RR, 73-45RR, 74-44BL, 74-54RR, and InVigor L130, InVigor L140P respectively). The six varieties were planted at two different target densities (120 and 30 plant m^{-2}) to determine whether phenotypic plasticity (i.e., increased branching) affects pod-retention resistance. Of the six varieties utilized in this experiment, three (named from hereafter Hyb1, Hyb 2 and Hyb3) were designated as more susceptible to pod shatter based on observations from the industry partners that supplied the seed, while the remaining three varieties (Hyb4, Hyb 5 and Hyb 6) were considered more resilient to pod shatter. Fertilization was performed according to the soil test analysis. At the 3-4 leaf stage, the appropriate in-crop herbicides were applied to control emerged weeds and at 50% flowering stage, the appropriate

fungicide for sclerotinia was applied, although this had little impact on the sclerotinia outbreak in the early seeding date experiment in 2014. Throughout the field season, the developmental stages of the canola varieties were recorded to account for any differences among the cultivars tested. At BBCH stage 84-85 (40 to 50% pod ripening, seeds dark and hard) before any pod drop or pod shatter had occurred, 2 meters row crop sample were collected to determine plant biomass and seed yield samples that were used to determine proportional (%) seed harvest losses.

CPT experiments (Obj. 2)

For this objective, the Canola Performance Trials (CPTs) used were entirely managed by the respective cooperator. In 2014, 18 different genotypes were used in the CPT experiments and data were collected at Carman, MB and Outlook, SK. In 2015, 19 varieties were included and data were collected at four different CPT experimental locations across Manitoba and Saskatchewan. Pod-retention resistance measurements were obtained for all varieties at the BBCH 78 developmental stage.

Pod-retention resistance

Method refinement experiments -Data for the pod retention resistance method were collected using a force gauge device at two different dates during pod and seed maturation. As the varieties had ± 2 days to maturity of difference, sampling was performed on the same day for all the varieties. The sampling schedule took place at the BBCH stage 78 (development of fruit 80%), and BBCH stage 85 (50% of pods ripe, seeds dark and hard). Result from the previous year showed no difference between measurements taken from the main *vs*. secondary rachis, so for field season 2014 it was decided to take measurements only from the distal (youngest) and proximal (oldest) position on the rachis position. For each rachis position a total of 15 measurements were taken from five different plants for a total of 120 measurements per each variety for experiments (15 measurements * two rachis positions * 4 replicates). In addition to that, average pod retention resistance measurements and average pod drop were calculated.

CPT experiments - Only one measurement at the BBCH 78 (development of fruit 80%) took place for determining pod-retention resistance in the CPTs among the varieties, either in 2014 or 2015. Data from our previous studies has indicated that relative differences in pod retention resistance among genotypes remain consistent throughout pod maturation.

Catch Trays

Method refinement experiments - At the early pod filling stage, two mesh-lined catch trays (76 cm x 15 cm) were placed in each plot, and before pod drop occurred, pods on the surrounding plants were marked lightly with different colors to determine the position on the plant from which the pods collected in catch trays originated. After BBCH stage 97 (plant dead and dry), catch trays were monitored weekly for pre-harvest losses and signs of predation. Trays were emptied only once at the end of the experiment to avoid contributing to pre-harvest losses by inadvertently manipulating the plants. Total pre-harvest seed losses (pod drop + pod shatter) in canola were recovered from the catch trays immediately before direct-harvesting the plots which was performed about three weeks after BBCH stage 97.

CPT experiments - Two mesh-lined catch trays (76 cm x 15 cm) were placed in each plot. Catch trays were placed in the plots during the filed season 2015, after authorization from the coordinator of the CPTs. Catch trays were removed at different dates depending by the harvest operation performed at each site year. For those site years that were direct-harvested, catch trays were removed from four to six days prior the operation, and for those site years that were swathed or pushed, trays were removed from two to four days before combining.

Statistical treatment of the data was a large part of this research and method development and refinement and as a consequence is described extensively throughout the results section.

Results

This project was comprised of two objectives. The first objective focused on refining the number of measurements and the plant location from where to obtain pod-retention resistance (PRR) measurements while the second objective focused on whether pod-retention resistance will lead to a better understanding of pod drop in canola. Although two distinct sets of experiments were used for each objective, there was significant overlap in the measurements between the experiments that allowed for comparisons among the studies. To address objective 1, we first determined the

number of sub-samples required to minimize the variation in PRR to obtain statistically sound and representative estimates of PRR without losing important information while minimizing the effort required for collecting PRR estimates. This was conducted using data collected in the 2013 method refinement experiments only and compared those results to data from a preliminary study conducted in 2012.

To determine the number of PRR measurements required to produce reliable estimates, standard errors of the mean were calculated for rachis type (main vs secondary) and rachis position (proximal vs. distal) in each experimental from the 25 measurements that were taken at each rachis type and rachis position. This preliminary analysis showed that about 12-15 PRR measurements per rachis type or rachis position were necessary to minimize the variation within treatment (Figure 1) and provide a statistically sound estimate of PRR that can be used for further analysis. Similar results were observed in a preliminary study conducted in 2012 (data not shown). This allowed us to confidently address the next parts of the objective which focused on determining the factors that affect PRR and their relative importance.

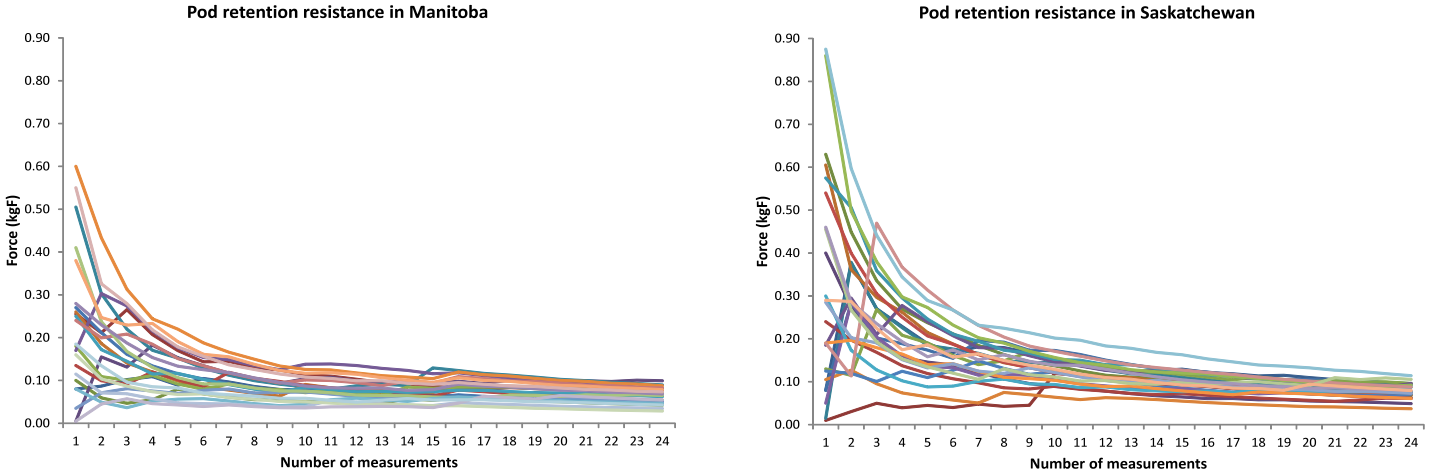

Figure 1. Standard errors of the means for increasing measurement intensities (from 2 to 25 measurements per plant). Only treatments with the highest and lowest standard errors are presented for clarity and to show the range among treatments in the 2013 Saskatchewan and Manitoba method refinement experiments.

Sources of variance for PRR, pod drop and pod shatter

To determine the relative importance of all factors and their interactions on PRR, pod drop and pod shatter, ANOVA was conducted on these response variables (Table 1). The factors investigated for PRR included genotype, density, position of the pods on the rachis (upper half vs. lower half), rachis type (main or secondary), developmental stage (time) of measurement, and location and year. For simplicity and due to limited replication within, location and year were combined into a single variable (site-year). We investigated the importance of rachis type (primary or secondary) in the 2013 field season only as we found that rachis type nor its interactions (combined contribution was less than 1.1% of the total variation) (data not shown) had no significant impact on the PRR measurements. Data from a preliminary study conducted in 2012 confirmed this as well (data not shown). Therefore, this treatment and source of variation was excluded from the experiments beginning in 2014. This reduced the number of PRR measurements that had to be taken without compromising any other part of the experiments.

The main variables that contributed to variation in PRR measurements included position on the rachis from which measurements were taken (28.4%), canola genotype (7.4%), site-year (5.5%), canola stand density (2.6%) and the developmental stage at which PRR measurements were taken (2.5%) (Table 1). Many of the interactions involving canola stand density were not significant and when significant only accounted for a small part of the total variation (3.3% for all interactions with density). As a result, this effect also was identified as a minor source of variation. The same was observed with many of the interactions involving the developmental stage at which the PRR measurements were taken (8.6% of the total variation partitioned among 15 different interactions). Greater partitioning of sums squares to the main effects for time of PRR measurement and canola stand density and limited partitioning of the sums squares to the interactions of these and all other variables indicated that only absolute values (main effect) in PRR measurement are influenced by these factors while relative values were largely unaffected. As a result, these factors appear of limited

importance when looking for differences in PRR among genotypes and site-years. The large contribution of rachis position to PRR indicates significant differences in PRR between proximal and distal plant parts. Similar to the more minor variables, all interactions among rachis position and all other variables only accounted for 9.1% of the total variation indicating an ability to condense the measurement regime without losing important information. The large contribution of variation from the main effect of rachis position from which measurement was taken is fortuitous as this source of variation can easily be controlled by the experimenter.

Table 1. Variance components (% of total variation), F-values and p-values for pod-retention resistance, seed loss via pod drop and seed loss via pod shatter for all factors and their interactions in the pod-retention resistance method refinement experiments. Type 3 p-values of significant effects are indicated in bold.

Factor		Pod-Retention Resistance			Pod Drop			Pod Shatter		
	df	% variation	F-value	p-value	% variation	F-value	p-value	% variation	F-value	p-value
SiteYear	3	5.5	2.1	0.1555	62.5	631.6	**<.0001**	55.4	168.5	**<.0001**
Genotype	5	7.3	30.8	**<.0001**	4.9	58.4	**<.0001**	26.0	326.6	**<.0001**
SiteYear*Genotype	15	1.7	2.4	**0.0020**	4.4	17.7	**<.0001**	5.2	22.0	**<.0001**
Density	1	2.6	54.8	**<.0001**	0.3	19.1	**0.0026**	0.4	27.7	**0.0122**
SiteYear*Density	3	0.5	3.3	**0.0214**	0.8	16.4	**<.0001**	0.0	0.5	0.9549
Genotype*Density	5	0.2	0.7	0.6639	0.4	4.6	**0.0508**	1.1	13.6	**0.0095**
SiteYear*Genotype*Density	15	0.7	1.0	0.5059	1.1	4.2	**0.0128**	1.7	7.2	0.0611
Rachis Position	1	28.4	594.4	**<.0001**	2.3	136.5	**<.0001**			
SiteYear*Rachis Position	3	1.6	11.4	**<.0001**	13.0	258.3	**<.0001**			
Genotype*Rachis Position	5	1.1	4.7	**0.0003**	0.4	5.1	**0.0322**			
SiteYear*Genotype*Rachis Position	15	0.9	1.2	0.2663	1.0	4.1	**0.0178**			
Density*Rachis Position	1	0.6	12.0	**0.0006**	0.0	0.1	0.8057			
SiteYear*Density*Rachis Position	3	0.2	1.4	0.2583	0.0	0.5	0.8752			
Genotype*Density*Rachis Position	5	0.1	0.4	0.8551	0.1	1.7	0.5466			
SiteYear*Genotype*Density*Rachis Position	15	0.2	0.3	0.9952	0.4	1.4	0.8022			
Time of Measurement	1	2.5	53.1	**<.0001**						
SiteYear*Time of Measurement	3	1.6	10.9	**<.0001**						
Time of Measurement*Genotype	5	0.9	3.8	**0.0022**						
SiteYear*Time of Measurement*Genotype	15	0.6	0.9	0.6015						
Time of Measurement*Density	1	0.3	5.7	**0.0170**						
SiteYear*Time of Measurement*Density	3	0.1	0.8	0.5103						
Time of Measurement*Genotype*Density	5	0.1	0.6	0.7303						
SiteYear*Time of Measurement*Genotype*Density	15	0.6	0.8	0.6669						
Time of Measurement*Rachis Position	1	2.2	46.5	**<.0001**						
SiteYear*Time of Measurement*Rachis Position	3	1.2	8.1	**<.0001**						
Time of Measurement*Genotype*Rachis Position	5	0.2	0.8	0.5652						
SiteYear*Time of Measurement*Genotype*Rachis Position	15	0.4	0.6	0.8980						
Time of Measurement*Density*Rachis Position	1	0.1	1.1	0.2899						
SiteYear*Time of Measurement*Density*Rachis Position	3	0.1	0.5	0.6818						
Time of Measurement*Genotype*Density*Rachis Position	5	0.0	0.0	0.9992						
SiteYear*Time of Measurement*Genotype*Density*Rachis Posit	15	0.3	0.4	0.9777						
Rep(SiteYear)	12	10.5	18.4	**<.0001**	0.41	0.98	0.4643	1.32	1.61	0.0959
Residual	561	26.8			7.98			8.84		

Pod drop measurements were not conducted on multiple measurement dates nor were dropped pods separated by rachis type (main vs. secondary). Marking pods to facilitate the ability to discern whether they dropped from the main or a secondary rachis was impractical. In this study with limited canola genotypes, however, it was practical to separate to mark the upper and lower portions of the rachis to discern from which half of the rachis the dropped pods originated. In a larger experiment, separation by rachis positon would also be too time-consuming. Pod shatter could not be separated by rachis position or rachis type and therefore variance was partitioned into even fewer components than pod drop. For both pod drop and pod shatter, site-year (environment) contributed most prominently to the differences observed in these measurements. Part of this could have been caused by differences in harvest dates among sites and years and likely also environmental conditions during pod filling and seed maturation. Similar to our observations in a previous study (Cavalieri and Gulden 2014), the importance of environment was greater for pod drop than pod shatter while the contribution of genotype to the total variation observed in pod drop (5%) was much lower than for pod shatter (26%) (Table 1). The divergence in significance of variance components between PRR and pod drop suggests a limited link between these two measurements. The proportion of variation for PRR and pod drop that was consumed by genotype and it's interaction with other factors, however, was surprisingly similar (12.4 vs. 12.7% of total variation) when accounting only for those factors that were the same among the measurements (i.e., excluding developmental stage of PRR measurement).

Treatment effects

Pod-retention resistance

The influence of the three most important factors (rachis position, site-year and genotype) affecting PRR is shown in Figure 2. The effect of canola stand density on PRR was not shown as density, similar to developmental stage of PRR measurement did not contribute to important interactions. On average, PRR was greater in low density stands compared to high density stands (0.9916 kg F vs. 0.8832 kg F) (data not shown) which was only a 12% difference in PRR, despite a 4-fold difference in seeding rates (Table 1), but does indicate some degree of plasticity in this trait. Interestingly, this effect was only significant among pods from the lower rachis position while plant density did not influence PRR among the upper pods. This difference was the cause of the significant interaction in pod-retention resistance between rachis position and stand density (Table 1).

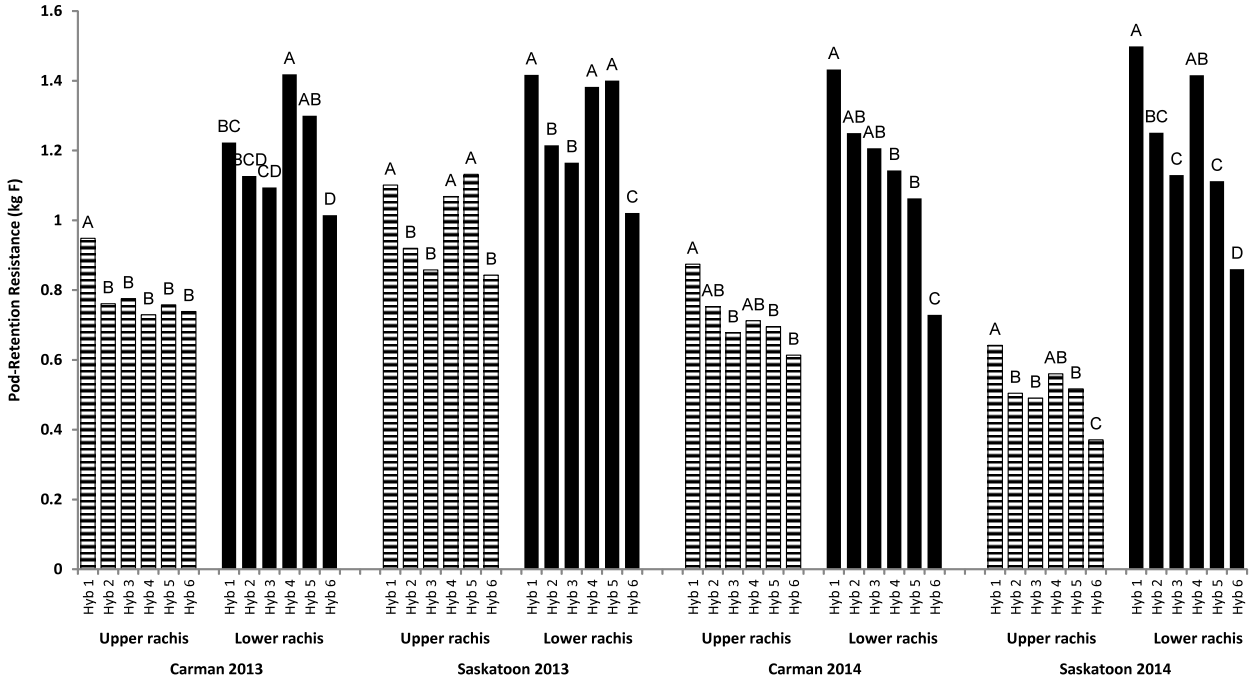

Figure 2. Pod-retention resistance for upper (horizontal lined bars) and lower (solid black bars) for each hybrid at each of the four site-years of the method refinement experiments conducted in 2013 and 2014. Within each site-year and rachis position, means with different letter are significantly different based on Fisher's protected LSD.

The force required to break the petiole was significantly greater for the lower section of the rachis compared to the upper part of the rachis (Figure 2). The combined analysis for the four site-years showed an average force of 1.12 ± 0.32 kg F required to break the petiole from pods from the lower rachis while 0.75 ± 0.03 kg F was required to break the petiole at the upper part of the rachis.

Pod-retention resistance measurements indicate relatively consistent behavior among genotypes at most of the four site-years (Fig. 2). Apparent differences were not always statistically significant at all site-years or rachis positions. A notable exception to this consistent behaviour was a lack of or reversal in trends in the relative pod-retention resistance between hybrids 3 and 4 for upper rachis measurements at Carman 2013 and at the lower rachis position at Carman 2014. Why this occurred is not known. Pod-retention resistance measured here appears to suggest no obvious link between visual pod shatter potential which is the characteristic by which these hybrids were selected and grouped (Hyb 1-3 vs. Hyb 4-6), which should not be unexpected as Cavalieri and Gulden (2014) showed that the relationship between pod drop and pod shatter may be limited. The PRR results indicate that as absolute values were quite consistent among site-years and relative differences among canola genotypes, at least for those chosen for this experiment, appear to be more variable among site-years, it is important to collect this information for a number of site-years and combining these data for an accurate estimate of PRR for individual canola genotypes. Differences among canola cultivars in the force required to break the petiole have been documented before (Hoseinzadeh et al. 2010). In that study, application of urea and stem moisture content also affected absolute pod-pulling force indicating plasticity in pedicel attachment and PRR.

Pod drop

Data collected from catch trays for the method refinement experiments (objective 1) were used to determine yield loss from pod drop and pod shatter. Of the six varieties used in this study, three (Hyb 1- 3) were considered as more susceptible to pod shatter while the other 3 were considered less susceptible to pod shatter based on observations from the industry partners that supplied the seed. Although single degree-freedom estimates showed higher pre-harvest seed losses via pod shatter (221.3 kg ha^{-1}) and pod drop (64.9 kg ha^{-1}) in the group of genotypes considered more susceptible to pod shatter, a great deal of overlap among genotypes within these purported groups was observed in pod shatter and pod drop and as a result, genotypes were not grouped for any of the analyses in these experiments. On average, pod drop (108.7 kg ha^{-1}) accounted for about 25% all pre-harvest seed losses while pod shatter (307.5 kg ha^{-1}) accounted for the remainder (data not shown). Analysis of pod drop variance components indicated that genotype and site-year were the most important factors influencing pod drop while density and all interactions with density contributed very little to pod drop and the results were summarized accordingly (Fig. 3).

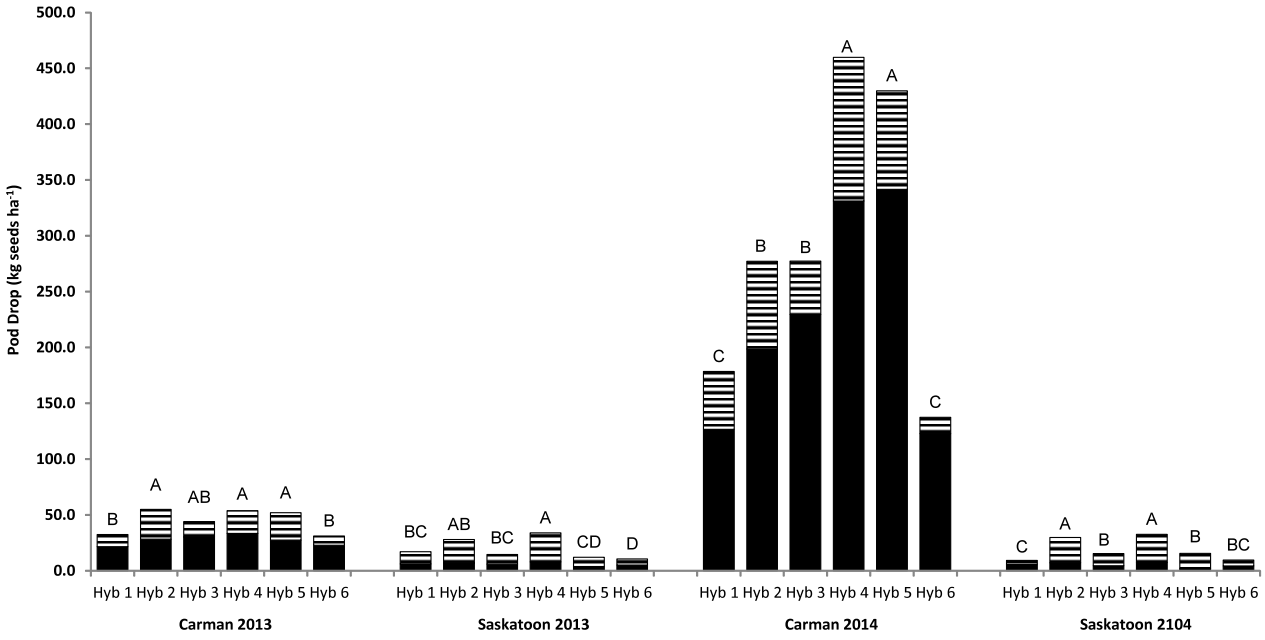

Figure 3. Total pre-harvest canola seed loss via pod drop from the upper (bars with horizontal lines) and lower (solid black bars) half of the rachis for each hybrid at each of the four site-years for the method refinement experiments conducted in 2013 and 2014. Means separation for total pre-harvest seed loss via pod drop is indicated. Within each site-year, means with different letters are significantly different based on Fisher's protected LSD.

Immediately noticeable was the strong effect of site-year (environment) on pod-drop (e.g., MB 2014) (Fig. 3). Delayed sampling due to inclement weather contributed to significantly greater pod drop in Manitoba in 2014 compared to all other site-years. The importance of delayed harvest to pod drop has been observed before (IHARF 2011). Despite this, trends in the relative differences among the cultivars were quite consistent among site-years with only minor changes in the relative ranking among the cultivars. A significant difference in the absolute amount of pod drop was also observed between rachis positions among the site-years. In Saskatchewan, pod drop tended to be greater from the upper half of the rachis, while in Manitoba on average, pod drop tended to be greater from the lower portion of the rachis. The cause for this is not known but may be related to differences in sampling times or subtle differences in sampling methods.

Variance components in the CPT experiments

The 2015 CPT trials contained 19 varieties providing a much larger range of genetic variation than the 6 selectively chosen varieties used in the method refinement experiments (Obj. 1). In addition, all CPT trials were harvested promptly. Pod drop was at the very early stages throughout and by the time of harvest, pod drop had not begun in all

experimental units. The 2014 CPT experiments contained 18 genotypes, but these differed from those in 2015 and therefore, the results could not be combined for analysis. In 2014, catch trays were not used in CPT experiments as these were swath harvested which may not be ideal for evaluating pod drop. As a result only PRR measurements were obtained in 2014. During the 2015 field season, catch trays were used in all four CPT experiments from which PRR measurements were collected. Two of the 2015 CPT experiments were direct-harvested, while one location was swath-harvested and at the last location canola plants were pushed prior to direct-harvest. The lack of significant interactions with genotype and location for seed loss via pod drop and weight of individual pods as well as the relatively weak interaction with genotype and location for total pod drop (entire siliques) showed that harvest method had a limited effect on pod-drop among genotypes and as a result all locations were combined for analysis (Table 2).

Table 2. Variance components (% of total variation), F-values and p-values for pod-retention resistance, seed loss via pod drop and seed loss via pod shatter for all factors and their interactions in the CPT experiments. Type 3 p-values of significant effects are indicated in bold.

Factor		Pod-Retention Resistance			Pod Drop			Pod Shatter		
	df	% variation	F-value	p-value	% variation	F-value	p-value	% variation	F-value	p-value
SiteYear	3	1.6	1.4	0.2811	22.4	33.4	**0.0001**	30.4	75.2	**<.0001**
Genotype	18	17.8	12.5	**<.0001**	8.5	1.9	**0.0202**	12.9	6.5	**<.0001**
SiteYear*Genotype	54	10.1	2.4	**<.0001**	12.9	0.9	0.1144	33.1	5.6	**<.0001**
Rachis Position	1	16.0	202.5	**<.0001**						
SiteYear*Rachis Position	3	5.3	22.2	**<.0001**						
Genotype*Rachis Position	18	4.5	3.2	**<.0001**						
SiteYear*Genotype*Rachis Position	54	5.3	1.2	0.1361						
Rep(SiteYear)	12	4.4	4.7	**<.0001**	2.7	0.9	0.5677	1.6	1.2	0.2651
Residual	443	35.0			53.5			22.0		

Despite the greater range in pod-retention resistance, variance component analysis for pod-retention resistance, pod drop and pod shatter in the 2015 CPT experiments showed similar results to those in the method refinement experiments (Table 2). Due to the larger number of varieties in this study, however, the variance partitioned to this effect was greater. Nevertheless, the relative distribution of the variance components among the three measurements remained similar to that found in the method refinement studies. As in obj. 1, variance for PRR partitioned into a large genetic component with only a small contribution of location, while the proportion of total variance partitioned to location was greater for the other response variables (pod-drop and pod shatter). Again, this illustrates a difference in sensitivity to environment between PRR and pod drop and suggests that a method for correcting for differences in absolute pod drop among site-years may be necessary to define a relationship between PRR and pod drop. In the CPT trials, part of this environmental effect may be related to the differences in the harvest methods and the timing of harvest. In contrast to Obj. 1, the location by genotype interaction consumed a much larger portion of the total variation for PRR in the CPT experiments indicating greater divergence in environmental influence among this broader group of genotypes.

The method refinement experiments showed that representative PRR measurements were best determined from data collected over several locations and that absolute pod-drop can vary substantially among locations. The same occurred among these CPT experiments where a significant location effect was observed in the absolute amount of pod drop (entire siliques) and the absolute amount of seed recovered from dropped pods (Table 3).

Table 3. Mixed model ANOVA output (p-values) for pod-retention resistance and pod drop parameter and canola yield for the main effects and their interactions for the 2015 CPT experiments. P-values of significant effects are indicated in bold.

		Pod-retention resistance				Pod Drop				Canola
		Force				Entire	Seeds	Individual	Proportion	Yield
Effect	df	Upper	Lower	Average	Specific	Pod	only	Pod	of Yield	
		(kgF)	(kgF)	(kgF)	(kgF g pod^{-1})	(kg ha^{-1})	(kg ha^{-1})	(g pod^{-1})	(%)	(kg ha^{-1})
Location	3	**0.028**	**0.0145**	0.3539	0.0795	**<.0001**	**0.0001**	**0.007**	0.1301	**<.0001**
Cultivar	18	**<.0001**	**<.0001**	**<.0001**	0.1704	**0.001**	**0.0202**	0.2103	**0.0222**	**0.0041**
Location*Cultivar	54	**0.0119**	**<.0001**	**0.0006**	**0.0416**	**0.0109**	0.1144	0.5004	0.6741	0.6012

Treatment effects in the CPT experiments

Pod-retention resistance

A much larger range in pod-retention resistance values was observed among the 18 genotypes in the 2014 CPT experiments (0.74 to 1.50 kg F) than in the method refinement study for Obj. 1 (0.78 to 1.05 kg F) (data not shown). Despite differences in the specific genotypes used in 2015, the range in PRR was similar among the four 2015 CPT experiments that were sampled was similar (0.95 to 1.68 kg F) to that observed in the 2014 CPT experiments.

In the 2015 CPT experiments, PRR differed among genotypes and these genotypic differences were not consistent among sites (Table 3). No location effect on PRR was noted in the 2015 CPT experiments. Similar results were observed for the 2014 CPT experiments where mean PRR and the differences in PRR among genotypes were the same at both locations and only a highly significant genotype effect was detected. In contrast to the results obtained for Objective 1, average PRR for the upper portion of the rachis (0.75 to 0.82 kg F) was more consistent among site-years than for the lower portion of the rachis (0.88 to 1.08 kg F) (data not shown). In the method refinement experiments, the site-year by rachis position interaction occurred via differences in the PRR (0.64 to 0.96 kg F) in the upper rachis position with no differences in the lower rachis position (1.06 to 1.19 kg F) (data no shown).

Pod drop

In the CPT experiments used for Obj. 2, pod drop also varied among locations although the same degree of divergence in pod drop among site-years was not observed. In addition, the interaction between location and genotype was significant as was the main effect for location (Table 3). In general, pod drop in the CPT experiments was much lower than that observed for the method refinement experiments (objective 1) and ranged from 0 to 44.7 kg ha^{-1} among all genotypes across all locations. The significant location effect was likely related to harvest method as less pod drop occurred at sites where the crop was swathed or pushed before direct-harvest. This suggests that pod drop may be a more significant source of harvest losses in direct harvested canola. In a side-by-side comparison, Haile et al. (2013) showed that total harvest losses in canola were similar between direct- and swath-harvested fields, but the source of the harvest losses was not identified in this study. The interaction observed among genotypes and locations for pod drop in the CPT experiments also was related to the different harvest methods as an analysis of the direct harvested locations only resulted in significant location (0.0001) and genotype (0.0477) effects, but no interaction between these two factors (0.9715).

Pod-retention resistance and specific pod-retention resistance correlations with pod drop

To determine if a relationship between pod drop and PRR exists, PRR was correlated against absolute pod drop collected from the catch trays for (a) all experimental units and (b) only those where pod drop was observed for the method refinement experiments and the 2015 CPT experiments (Table 4). Correlations using all experimental units including those where no pod drop was observed were not successful (data not shown). There was no obvious relationship between PRR and those experimental units where no pod drop was observed. All reported correlations excluded experimental units where no pod drop was observed. For the CPT experiments, upper, lower and average PRR was correlated with total pod drop, as the region of the rachis from which pods dropped could not be identified in these experiments. For the method refinement studies, pods were marked with colour and therefore, upper, lower and total pod drop were correlated with upper, lower and average pod-retention resistance, respectively. Correlations were conducted on total dropped pod weight, i.e. the unopened siliques rather than the seed component only. These correlations proved more successful (data not shown) in part because PRR acts on entire siliques and not only their seed component. This also precluded determining pod drop as a proportion of yield as the total weight of entire, seed-filled siliques is not known. The simplest approach for elucidating a relationship was to correlate the average PRR for each experimental unit with absolute pod drop for that experimental unit. Unfortunately, the correlations of PRR and absolute pod drop were poor, non-significant and the low Pearson R values were inconsistent in direction (Table 4). No obvious improvements were found when separating the position on the rachis from which the force measurements were obtained (upper, lower, average). Converting the seed portion of absolute pod drop to a proportion of yield also did not improve the correlations, again indicating no relationship between PRR and pod drop.

For the correlation on the combined data for the method refinement study (Obj. 1), the large differences in pod drop among site-years (e.g. Carman 2014 vs. the rest) had to be normalized as without correction, the pod drop differences among site-years dictated the direction and significance of the correlations. To accomplish this, the pod drop data

(upper, lower and total) for each site-year were centered around a mean of 10 with a standard deviation of 1. Centering the mean at 10 rather than the more traditional center of 0 facilitated log transformation which was necessary to improve the correlations. Similarly, PRR (upper, lower and average) was standardized and log transformed. Log transformation of these variables proved valuable, however, standardization to the same mean for each location was not necessary for PRR.

Table 4. Correlations between absolute pod drop (entire siliques) and pod-retention resistance for the CPT and method development experiments. Only experimental units where pod drop ocurred are included. Peasron R correlation coefficients and p-values of significant correlations are indicated in bold.

CPT Experiments 2015 (Obj. 2)

Total pod drop correlated with average pod-retention resistance after averaging replicates at each location.

		Upper rachis		Lower rachis		Average	
	n	Pearson R	p-value	Pearson R	p-value	Pearson R	p-value
Combined	73	-0.05	0.6561	-0.02	0.8379	-0.05	0.6584
Thornhill	19	-0.42	0.0766	-0.18	0.4540	-0.36	0.1321
Elm Creek	17	0.04	0.8772	0.00	0.9957	0.03	0.9025
Waka	19	-0.37	0.1171	-0.19	0.4420	-0.35	0.1455
Saskatoon	18	-0.19	0.4521	0.30	0.2263	0.00	0.9943

Total pod drop correlated with average specific pod-retention resistance after log transformation transformation of both variables and averaging replicates.

		Upper rachis		Lower rachis		Average	
	n	Pearson R	p-value	Pearson R	p-value	Pearson R	p-value
Combined	73	-	-	-	-	**-0.81**	**<.0001**
Thornhill	19	-	-	-	-	-0.06	0.8213
Elm Creek	17	-	-	-	-	**-0.81**	**<.0001**
Waka	19	-	-	-	-	**-0.59**	**0.0078**
Saskatoon	18	-	-	-	-	**-0.94**	**<.0001**

Method Refinement Experiments (Obj. 1)

Pod drop for each section and combined correlated with pod-retention resistance standardized among locations.

		Upper rachis		Lower rachis		Average	
	n	Pearson R	p-value	Pearson R	p-value	Pearson R	p-value
Combined	165	0.11	0.1580	**0.18**	**0.0175**	**0.18**	**0.0229**
Carman 2014	44	-0.09	0.5800	**0.33**	**0.0228**	0.19	0.2156
Saskatoon 2014	45	0.24	0.1165	0.03	0.8375	0.19	0.2630
Carman 2015	45	0.03	0.8553	-0.02	0.9082	-0.03	0.8332
Saskatoon 2015	43	**0.45**	**0.0041**	**0.41**	**0.0129**	**0.56**	**0.0011**

Pod drop for each section and combined correlated with specific pod-retention resistance and standardized among locations after log tranformation of both variables.

		Upper rachis		Lower rachis		Average	
	n	Pearson R	p-value	Pearson R	p-value	Pearson R	p-value
Combined	159	-0.04	0.5690	**-0.30**	**0.0001**	**-0.23**	**0.0029**
Carman 2014	44	-0.23	0.1273	**-0.55**	**<.0001**	**-0.33**	**0.0293**
Saskatoon 2014	45	0.06	0.6931	-0.31	0.0623	-0.04	0.8065
Carman 2015	45	0.03	0.8301	-0.23	0.1333	**-0.32**	**0.0344**
Saskatoon 2015	43	-0.04	0.8220	-0.03	0.8634	-0.20	0.2840

Specific pod-retention resistance

Specific pod-retention resistance (SPRR) is the average PRR determined for a treatment during pod maturation divided by the average weight of individual dropped pods from that treatment. Specific pod-retention resistance (kg F g pod^{-1}) provides an indication of relative strength of attachment of the dropped pods and cannot be determined when no pod drop is measured. At the time PRR measurements are obtained, pods are still filling and the weight of pods may not be reflective of final pod weight, nor does it provide information on the dropped pods. With SPRR, average the correction for pod weight removes the effects of pod size and provides a more direct measure of pod attachment. This will allow us to examine potential explanations for the differences in the amount of dropped pods among treatments. A lower SPRR indicates that dropped pods were attached less strongly per unit dry matter than pods with a high SPRR. One would expect that absolute pod would be greater in treatments were SPRR was lower.

For the CPT experiments, correlation analysis between SPRR (kg F g pod^{-1}) and absolute pod-drop proved highly successful (Table 4). Despite significant differences in pod drop among locations (Table 3), there was no need to standardize the means among locations. A close relationship between average individual weight of dropped pods across all genotypes at a location and yield differences among locations indicates that this correction likely also removed location specific effects that standardization would have removed (Table 5). The weight of individual dropped pods varied about 3-fold among locations and roughly mirrored average yield at each location, but surprisingly was not different among the 19 genotypes in this study (Table 3). Despite the lack of genotypic differences in weight among dropped pods, correcting PRR with average individual weight of dropped pods proved highly successful in highlighting a contributing factor to differences in pod drop.

Table 5. Mean yield and iondividual pod weight for each location of the 2015 CPT experiment. Means followed by different letters are significantly different based on Fishers protected LSD.

Location	Canola Yield	Individual pod weight
	(kg ha^{-1})	(g pod^{-1})
Thornhill	2760.4 B	0.067 AB
Elm Creek	2293.8 C	0.035 C
Waka	3876.6 A	0.093 A
Saskatoon	2405.7 C	0.051 BC

Using log transformed measurements for both variables improved linearization of the data and averaging the replicates at each location increased the Pearson-R correlation coefficient to -0.81 (p-value < 0.0001) when all sites were combined in the analysis (Table 3). The correlations also were highly significant within all (p-values <0.0078) but one location (p-value = 0.8213) and all correlations were clearly in the same direction. For the highly significant locations, the Pearson R values were similar or greater than for the combined analysis, however, for the Thornhill, MB location, the Pearson-R value was low. Reasons for this are unclear as this location was direct-harvested in a similar timeframe and pod drop was observed in many experimental units. Again, this shows the need for multiple locations for the generation of meaningful data that overcomes the at times significant and as yet unexplained site-specific effects on pod drop.

Within genotypes over all locations, the mean absolute weight of dropped pods ranged from 0 to 19.7 kg ha^{-1} while mean SPRR ranged from about 0 to 69.6 kg F g pod^{-1}. Mean PRR, on the other hand, ranged from 0.95 to 1.69 kg F. As highlighted in the variance component analysis, the relative variation among locations was greatest for pod drop and less for PRR and SPRR (Fig. 4). Determination of SPRR (Fig. 4 bottom), however, changed the relative ranking and in some case the degree of variation associated with location among many of the canola genotypes when compared to PRR (Fig. 4 middle). The changes in relative ranking and degree of variation within genotypes across locations appear to have contributed to the substantial improvement in the correlations with pod drop. No differences in average individual weight of dropped pods among cultivars was observed (Table 3) which suggests that cultivar specific preferential drop of pods based on individual pod weight throughout the evaluation period likely did not occur. Specific pod-retention resistance as a response variable in ANOVA resulted in the loss of significance among genotypes and greatly reduced the significance of the genotype by location interaction which was observed for PRR (Table 3).

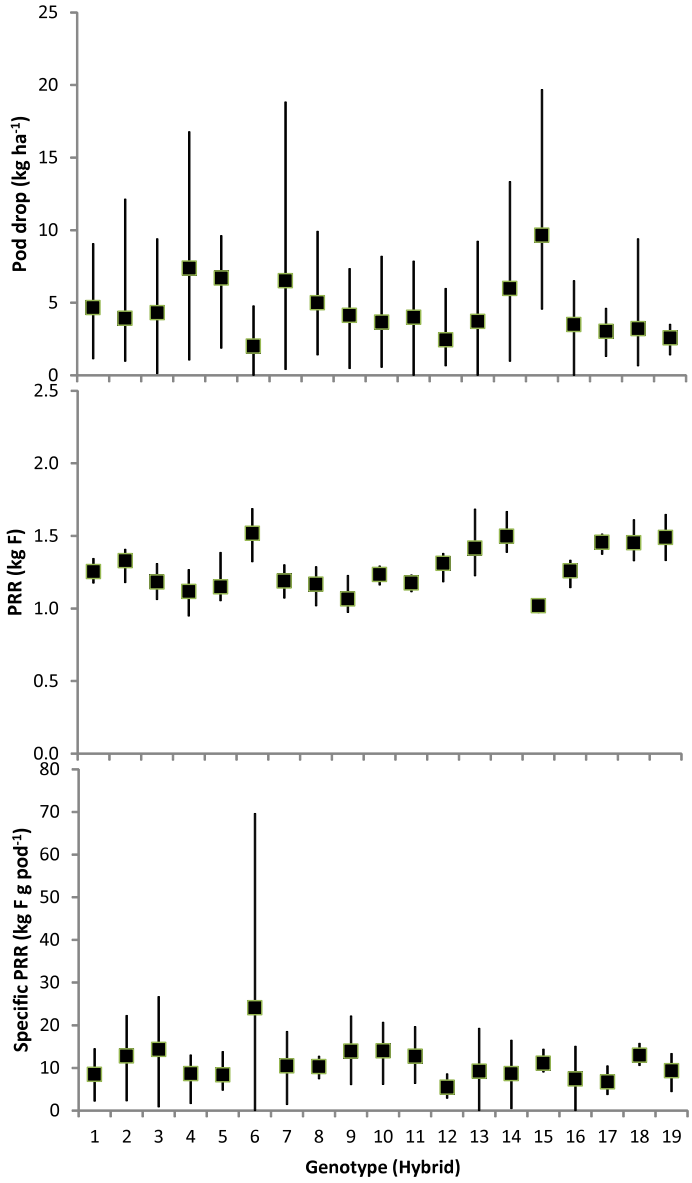

Figure 4. Mean (black square markers) and range (lines) of location means for pod drop (top), pod-retention resistance (PRR – middle), and specific pod-retention resistance (SPRR – bottom) for each genotype used in the 2015 CPT experiments. Bar ends indicate the maximum and minimum location means (average for the four replicates at each location) for each genotype and thereby define the range of location means used for correlation analysis.

Correlations in the Method refinement experiments

After standardization and log transformation, a significant correlation also was observed between absolute pod-drop and specific pod-retention resistance in the pod-retention method refinement is experiments with only 6 canola genotypes, but only when all observations were used in the analysis (Table 4). When the correlation was conducted on average values for each site as for the CPT experiments, there appeared to be insufficient replication (n=24 (combined) and n=6 (individual locations)) for any of the correlations to be significant (data not shown).

Although the overall correlation was significant (p-value = 0.0027), the correlation coefficient was relatively low (Pearson R = -0.236) (Table 4). A low Pearson R-value here should, however, not be surprising given the many sources of variation that were identified in the variance component analysis (Table 1). Standardization of these data only removed main effect variation of location, but does not account for variation partitioned to the interactions or other main effects. Given the importance of environment in pod-drop and that standardization among site-years (environments) cannot remove all environmental variation, a low Pearson R is not unexpected. Among the locations, Pearson R-values for this correlation were greater at Manitoba 2013 (-0.32) and Manitoba 2014 (-0.33) and significant and trended in the same

direction at Saskatchewan 2014 and 2015, where they were not significant (Table 4).

Conclusion

In summary, this project highlighted the contribution of key factors and their relative importance to pod-retention resistance and pod drop and showed a clear relationship between specific pod-retention resistance and absolute pod drop. Dropped pods from canola genotypes where low pod drop was observed required on average a higher force per gram pod to dislodge from the plant than dropped pods from plants with higher yield losses from pod drop. Despite several factors contributing in different order of importance to variation in pod drop (location, rachis position and genotype) and pod retention resistance (rachis position, cultivar and location), this highly significant relationship was found. Specific pedicel attachment strength appears to play an important role in pod drop and requires further investigation. For example, it is not known how environmental factors during seed maturation contribute to this relationship or how plasticity in pod size and pod-retention resistance interact. The strong correlation discovered here suggests a significant component to pod drop appears to be heritable and could be exploited by canola breeders to reduce canola harvest losses due to pod drop.

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4. Significant Progress/Accomplishments

This project resulted in significant improvement in the understanding of yield losses due to pod drop in canola. Major findings and developments of this project included:

i) Development and refinement of a method that can be used to measure pod-retention resistance in *Brassica napus* canola
ii) Important variance components that contribute to pod-retention resistance (rachis position, cultivar and environment (location)), pod drop (location, rachis position and genotype) and pod shatter (environment (location) and genotype) were separated from minor variance components for pod-retention resistance (rachis type, developmental stage of measurement, canola stand density), pod drop (canola stand density) and pod shatter (canola stand density)
iii) Despite the many factors and different levels of importance by which they contribute to variation in pod-retention resistance and pod drop, a highly significant relationship between average specific pod-retention resistance and total pod drop was identified.

The identification of the relationship between specific pod-retention resistance and pod drop is new and provides important insights into this environmentally sensitive mechanism for harvest losses in canola. The identification of this relationship indicates that specific pod attachment strength is an important factor contributing to differences in pod drop among genotypes. This is encouraging in that it suggests that breeding efforts may be able to reduce limit this potential source of harvest losses in canola. More research on better understanding the factors that contribute to pedicel attachment strength is necessary to improve our understanding of pod drop in canola.

5. Research and Action Plans/Next Steps
Refer to the two previous sections.
6. Budget impacts in the event major issues or variance between planned and actual is noted:
No major issues occurred.

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