

# **PROJECT DETAILS**

- Title: Developing methods to estimate pod drop and seed shatter in canola
- **Funders:** Agriculture and Agri-Food Canada, Alberta Canola, Canola Council of Canada, Manitoba Canola Growers and SaskCanola
- Research program: Growing Forward
- Principal investigator: Robert (Rob) Gulden
- Collaborators/additional investigators: Neil Harker, Linda Hall, Steve Shirtliffe, Christian (Chris) Willenborg
- Year completed: 2013

# **Final report**

Canola is the major oilseed crop grown in western Canada. Canola also is a crop with high potential for seed-shatter and pod-drop which at harvest can be substantial (Gulden et al. 2003a, Gan et al. 2008). Seed-shatter and pod-drop are both agronomic and economic issues. Pod-drop and seedshatter vary among genotypes and are influenced strongly by environmental conditions (Price et al. 1996, Pekrun et al. 1998). Average canola harvest losses 5.8% of yield have been reported, which in terms of viable seeds means about 3,000 seeds m-2 (Gulden et al. 2003a, 2003b). In a larger cross-prairie on-farm survey conducted as part of the GF1 Science-Cluster, initial results indicated that proportional harvest losses are similar today, but due to higher canola yields, absolute harvest losses have increased substantially in the past decade (Gulden et al. 3.4.1). Gan et al. (2008) reported similar harvest losses for a canola genotype grown in a small plot study at several locations in Saskatchewan from 2004-2006. In a recent study in China, where canola is often still hand-harvested, similar results were reported, where harvest losses in canola were high while the plants were drying in the field and increased when harvest of the crop was delayed (Zhu et al. 2012). Traditionally, direct-harvesting canola has not been recommended because the risk of yield loss due to shattering overshadowed the potential benefits (Holzapfel et al. 2010). As direct combining becomes more prevalent, the need for pod-drop and seed-shatter resistant canola varieties increases. Over the past four decades, a number of methods have been developed for assessing shatter resistance in canola (e.g., Josefsson 1968, Gan et al. 2008, reviewed in Kadkol et al. 1983), but many of these have not been compared directly (e.g., Wang et al. 2007). Josefsson (1968) developed a visual rating scale that has been used in other studies (Kadkol et al. 1982, Wang et al. 2007), but this method has not been compared to catch trays used recently by Gan et al. (2007). At this time, however, no effective and proven tools to estimate pod-drop and seed-shatter accurately and consistently are available. An increase in individual seed size in hybrid canola varieties compared to open-pollinated varieties has been documented (Elliott et al. 2008). Few data are available on whether this increase in individual seed size affects pod-drop and seed-shatter between these canola breeding types.

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### Objectives

In this study different methods (catch tray, vacuum, pod retention resistance and visual rating) were evaluated to address the following questions:

How do the visual rating and tray based methods compare for determining pod-drop and seedshatter?
Can digital images be used to estimate pod-drop and seed-shatter?

3) Can pod retention resistance be quantified quickly and reliably and is this measurement related to pod-drop in canola?

4) Are there fundamental differences in pod-crop and seed-shatter between open-pollinated and hybrid canola varieties?

**Site Description** - Field trials were conducted in 2011 and 2012 at two different locations in Manitoba; at Carman (Ian N. Morrison Research Farm) and at Kelburn (Kelburn Richardson Research Farm). Carman is in the Black Soil Zone, has an average annual precipitation of 517 mm and a mean annual temperature of 3.2 °C. Kelburn is in the Black Soil Zone, has an average annual precipitation of 664 mm and a mean annual temperature of 4.9 °C. The locations are about 75km apart.

**Design and Treatments** - Eight different canola varieties (four hybrid and four open pollinated) were seeded at two target densities (40 and 120 seeds m-2) for a total of sixteen treatments. The cultivars evaluated were Q2 (open pollinated), 46A65 (open pollinated), InVigor 5440 (hybrid - Bayer CropScience), InVigor L130 (hybrid - Bayer CropScience), 45H29 (hybrid - Pioneer Hi-Breed), 45S52 (hybrid - Pioneer Hi-Breed), Rugby (open pollinated - SeCan), and Café (open pollinated - SeCan). The experimental design was a randomized complete block design (RCBD) with four replicates.

**Crop Management** - In 2011 experiments were conducted at Kelburn (seeding dates June the 1st and the 16th) and Carman (seeding date June the 29th). Field experiments were repeated in 2012 at Kelburn (seeding dates May the 9th and 24th) and Carman (seeding dates May the 14th and June the 4th). Before planting, the experiments were fertilized to recommendation. Canola was seeded using a 2 m wide, double-disc, small plot seeder with 15 cm row spacing. Seeding rates were adjusted for the size of the canola seed with a targeted plant density of 40 and 120 plants m-2. At the 3-4 leaf crop stage, a tankmix of sethoxydim (500 g ai ha-1), ethametasulfuron-methyl (15 g ai ha-1) clopyralid (151 g ai ha-1) and the adjuvant Merge (0.5% v/v) was applied in-crop to control emerged weeds. At harvest, an area 1.5 m x 7 m was straight cut using a Kincaid 8xp, small plot combine *Seed shatter and pod-drop methods* 

**Catch tray method** - This method was used to determine pod-drop and seed-shatter in canola before harvest. Two mesh-lined catch trays (76 cm x 15 cm) were placed in each plot at the early pod filling stage (Fig. 1). Samples were recovered from the catch trays immediately before direct-harvesting the plots. After the collection, the samples were divided into shattered seed and pod-drop (full pods) fractions. The number of full pods was counted before hand-threshing to determine the harvest loss due

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to pod-drop. Seed-shatter and pod-drop were expressed in weight (kg ha-1) and as a percentage of total yield.



**Fig. 1**. Trays used to determine pod-drop and seed-shatter in canola before harvest. In 2012, catch trays were modified by adding a top section to reduce possible predation and / or the effect of adverse weather conditions.

**Vacuum method** - This method was used to determine total canola harvest loss (combination of preand post-harvest). This method used a wet–dry vacuum cleaner to collect samples that included crop residue, non-harvested seeds, and some soil from six 25 by 25 cm2 quadrats chosen randomly within each plot. Canola seeds in these samples were recovered as described by Gulden et al. (2003a) and data were expressed as weight of seeds (kg ha-1) and as percentage of total yield.

**Pod-retention resistance** - This method was pioneered in this study as a potential method to estimate pod-drop in canola. The method provided a quantitative pod-retention resistance measurement determined using a digital force gauge (Digital Push Pull Gauge, Model: SHWSFG102, Shimana, Fig. 2). Data were collected from a number of different pods at designated plant positions (main rachis or branch) and rachis portions (middle of the top half or middle of the bottom half). The data represented the force required to break the pedicle of the pod from the rachis, the weakest point of attachment of the pods. Pods on which pod-retention resistance was measured were collected to determine total pod weight and seed weight in each pod. In the field study, pod-retention resistance and pods were collected

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one week before combining and on the day before combining the crop. In a separate greenhouse experiment conducted throughout the winter, pod-retention resistance and pods were collected at 5 weekly time intervals immediately before harvest. In both studies, the moisture content of individual pedicles, the small 'stem' that connects the pod to the rachis, was measured to determine whether this affects pod-retention resistance. Pod weight was used to normalize the quantitative data of the force gauge expressed in kg force (kgF g pod-1).



Fig. 2. Digital force gauge tool in action in the field.

**Visual rating** – This method is based on the visual estimation of seed-shatter in a standing canola crop. The estimated percentage of shattered pods that were still attached to the plant in each plot was rated independently by two researchers immediately before harvest (the same time the catch trays were removed) and the data collected were averaged for each plot (Fig. 3).





**Fig. 3.** Examples of the visual estimation of seed-shatter in two varieties. The percentage of shattered pods that were still attached to the plant was rated 70% (left) and 5% (right).

**Image analysis** - Image analysis was used to attempt to develop a rapid method for estimating pod-drop and seed-shatter in canola. Images of canola seeds and pods (in 25 by 25 cm quadrats) were collected both in the greenhouse and in the field using a digital camera on a. The images were subjected to image analysis using *ASSESS 2.0* software (APS Press, American Phyto-pathological Society, St. Paul, MN). *Data Collection and Analysis* 

Data were collected throughout the growing season including plant emergence, initial flowering date, plant height, total biomass, canola yield and the various pod-drop and seed-shatter measurements described above. The yield samples used to determine proportional (%) harvest losses were collected at physiological maturity before any pod-drop or seed-shatter occured. After threshing, yield samples were cleaned, weighed and corrected to 10% seed moisture content. Yield was expressed in kg ha-1. Data were analyzed using the Mixed procedure in SAS. Cultivar, density, location and their interactions were considered fixed effects and blocks within location were designated as the random effect. All data were tested for normal distribution of residuals and outliers were removed using Lund's test when necessary. The data also were tested for heterogeneity of the variance among treatments and the model was adjusted to account for this when necessary. To determine the differences between open-pollinated and hybrid cultivars a single-DF estimate was constructed. Correlation analysis was used to compare the four methods.

# Results

Due to extremely poor and variable plant emergence at Carman in 2011, this site had to be abandoned and only Kelburn results are presented for 2011. Seed yield data, used to determine

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proportional harvest losses for the eight varieties in this study, varied substantially among the varieties and site-years. Plant density did not affect any of the response variables in this study and therefore the data were combined throughout. In the low density treatment, plant stands tended to be variable and greater than the target of 40 plants m-2 which may have contributed to the lack of a density effect. Statistical analysis of the data collected during the two years of these field experiments showed a highly significant effect (p < 0.0001) for cultivar and in the interactions of cultivar by year and cultivar by location; consequently, data are presented within location and year (Fig. 4).



**Fig. 4** Total yield of 4 hybrid (Hyb solid bars) and 4 open-pollinated (OP - pattern bars) canola varieties at Kelburn 2011 (black), Kelburn 2012 (white), and Carman 2012 (grey). Within year and location, means with different letters are significantly different based on Fisher's protected LSD. Standard errors of the means and significance of single degree-freedom contrasts comparing the breeding-types are indicated.

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In 2011, seed yield at Kelburn ranged from 1320 to 1857 kg ha-1 (Fig. 4). The contrast Hyb vs. OP was statistically significant (p = 0.0158), showing that the group of hybrid varieties produced a higher yield than the openpollinated varieties. The average yield of the two best-yielding open-pollinated varieties was not significantly different from that of the two lowestyielding hybrids varieties.

In 2012 at Kelburn, seed yield ranged from 1359 to 1998 kg ha-1 and was similar to the yield range observed in the previous year (Fig. 4). In 2012, however, the contrast between the two canola breeding-types was not significant (p = 0.6793).

In 2012 at Carman, canola yields ranged from 1304 to 2421 kg ha-1 (Fig. 4). Similar to Kelburn 2011, the yield of the hybrids was significantly greater (465 kg ha-1) than that of the open-pollinated varieties. At this location, greater yields in hybrids were primarily influenced by Hyb3 and Hyb4 which produced a significantly greater yield than any of the openpollinated varieties. The yields of Hyb1 and Hyb2 were similar to that of the open-pollinated varieties.

**Catch tray method** - Catch tray was the only method evaluated that allowed for the simultaneous, independent determination of poddrop and seedshatter. In this study, catch trays were used to determine pre-harvest yield loss only. The late harvest precluded reinsertion of the catch trays immediately before harvest without causing some harvest losses. Significant differences among the individual varieties and the two breeding-type groups were observed in the absolute and proportional total pre-harvest seed losses; however, these were not always consistent

between the two years or locations (Fig. 5). At Kelburn in 2011 and 2012, catch trays





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indicated significantly greater absolute preharvest seed losses in hybrid compared to openpollinated cultivars, however, differences in proportional seed losses were not observed between these breeding-types (Fig. 5 top and middle). This indicates that the difference in absolute seed losses between the breeding-types was clearly due to the differences in yield.

Interestingly, the varieties that contributed to different seed losses between the breeding-types at Kelburn were not the same in 2011 and 2012 (i.e., Hyb 4 and OP1).

At Carman in 2012, a difference in both absolute and proportional pre-harvest seed loss was observed between the canola breeding-types (Fig. 5 bottom). Removal of Hyb2 from the analysis, which appeared to be the most influential hybrid variety, did not change the significance of the estimate in absolute pre-harvest seed losses between the breeding-types (estimate value before and after removing Hyb2 was 259 and 168 kg ha-1, respectively). After removal of Hyb2, proportional seed losses due to seed shatter and pod-drop were no different between the breeding-types.

The catch tray method also allowed for the evaluation of pod-drop and seed-shatter independently. It could be assumed that the inherently larger seed size of hybrid canola varieties may be contributing to increased pod-drop in lieu of seed-shatter and this method allowed us to test this hypothesis by comparing the proportional contribution of seed shatter to total pre-harvest seed losses. There was no evidence that hybrid varieties were more prone to pod-drop than seed shatter. In fact, the results from this study indicate the opposite in that when significant differences were observed between varieties or breeding-types (Fig. 6), seed shatter was greater in hybrids than OPs. There were, however, differences in the relative contribution of seed shatter to yield loss among varieties within breeding–type that were consistent over the three site years indicating a strong genetic component to the propensity of seed shatter and pod-drop within varieties (e.g. Hyb2, Hyb3, and OP2). A genetic component to seed shatter has been observed before (Morgan et al. 2000, Gan et al. 2008).

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**Fig. 6** Seed shatter (as a proportion of total seed in catch trays) of 4 hybrid (Hyb solid color) and 4 openpollinated (OP - pattern color) canola varieties at Kelburn 2011 (black), Kelburn 2012 (white), and Carman 2012 (grey). Within year and location, means with different letters are significantly different based on Fisher's protected LSD. Standard errors of the means and significance of single degree-freedom contrasts comparing the breeding-types are indicated.

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**Vacuum method** - The vacuum method was used to evaluate total harvest losses immediately after harvest. This method cannot be used to distinguish between pod-drop and seedshatter, but is considered to be accurate due to the relatively large area that can be sampled in a short time compared to the catch tray method. Similar to the catch tray method, significant differences in the absolute and proportional total harvest losses were observed among the two breeding-types and groups and varieties (Fig.7).

In contrast to total harvest losses determined using the catch tray method, absolute and proportional yield loss was greater in the hybrids compared to the open pollinated varieties at all locations and in all years. For most varieties, pre-harvest yield losses determined using the catch tray method agreed well with total pre- and post-harvest losses determined using the vacuum method. The absolute differences between the pre-harvest tray and post-harvest vacuum method are relatively small indicating that a large portion of the total harvest losses occurred before harvest. These experiments were direct-harvested at a very late stage to be able to determine differences in pod-drop and seedshatter among varieties and therefore it is not surprising that most of the harvest losses were incurred before harvest. In OP1 at Kelburn in 2011, pre-harvest catch tray losses (Fig. 5 top) were greater than post-harvest vacuum losses (Fig. 7 top). It is not clear why this was observed, but it could be related to spatial variability and the small sampling area associated with the catch tray method. This, however, was only observed in OP1 at Kelburn 2011. Based on pre- and postharvest losses, the data suggest that there were differences among the varieties in the amount of seed that was lost during the harvest process.

For example, Hyb3 and Hyb4 at Kelburn and Carman 2012 appeared to exhibit greater losses during harvest than the other varieties (Fig.7 middle and bottom). This, however, was not consistent among all siteyears and in a separate analysis of the statistical

differences was not significantly different from other varieties.



and location, means with different letters are significantly different based on Fisher's protected LSD. Standard errors of the means and significance of single degree-freedom contrasts comparing the breeding-types are indicated.

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**Visual rating** – Seed-shatter, particularly from siliques (pods) that are split-open while still attached to the plant, is relatively easy to rate visually in canola. This is because of the clear difference in the colour of pods where the distinct, white septa (membranes between the pod halves) are exposed compared to the tan colour of intact pods. In general, visual rating of seed-shatter agreed well with seed-shatter results using catch trays. As confirmed with the quantitative catch tray data, the visual seed shatter rating of Hyb2 also was substantially greater than other varieties (Fig. 8). Removal of this variety from the hybrid group had no effect on the higher seed-shatter rating for hybrids compared to OP varieties at two of three locations, although the absolute value of the difference decreased from 13 % to 4 % in Kelburn 2011 and 18 % to 9 % in Carman 2012. At Kelburn 2012, however, removal of this hybrid resulted in no difference between the breeding-types (13% when Hyb2 was included). Within site year, the relative seed-shatter rating stended to be similar among the varieties over the three site years indicating that visual seed-shatter rating was a relatively effective and robust measurement. Visual rating also was the most time-efficient measurement. Unfortunately, this rating provides no information about pod-drop. In the greenhouse we observed that pod-drop occurs virtually exclusively at the rachis, leaving no immediately obvious mark by which to rate this phenomenon.



**Fig. 8** Seed shatter estimated by visual ratings of 4 hybrid (Hyb - solid bars) and 4 open-pollinated (OP - pattern bars) canola varieties at Kelburn 2011 (black), Kelburn 2012 (white), and Carman 2012 (grey). Within year and location, means with different letters are significantly different based on Fisher's protected LSD. Standard errors of the means and significance of single degree-freedom contrasts comparing the breeding-types are indicated.

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Pod retention resistance – As a method to potentially estimate or predict pod-drop, this method was employed at different times and pod moisture contents during pod ripening. Data were collected with the force gauge from the main rachis and branches (plant position) and separately from the top half or the bottom half of each rachis (rachis position). The force data represents the force required to break the petiole of the pod at the rachis, the weakest point of attachment of the pods. During the 2011 field experiments and the supplemental greenhouse experiments, pods were collected throughout pod ripening to determine whether pedicel moisture content influenced the pod-retention measurements over time. Both studies clearly indicated no relationship between pedicle moisture content and pod-retention resistance (kgF) nor did these studies show a difference in pod-retention resistance measurement over time (data not shown). These results indicate that pod-retention resistance is not affected by time, or moisture content, making it an attractive potential candidate for high-throughput applications. There also was no difference in pod-retention resistance between measurements taken from the main rachis and branches; however, pod retention-resistance was always greater for pods located in the bottom half of the rachis compared to the top half of the rachis. As there was no interaction between rachis position and other variables, all force gauge data were combined to represent an average value for all pods on the plant.

Overall, pod-retention resistance was remarkably consistent among varieties over years and locations (Fig. 9). Hybrid varieties consistently exhibited greater pod-retention resistance than openpollinated varieties. The estimated difference between the breeding-types was quite consistent among years and locations and ranged from 0.13 to 0.16 kgF. This represents about a 10 to 25% increase in pod-retention resistance and likely contributes to explaining the higher contribution of seed-shatter to total pre-harvest seed losses observed in the catch trays for the hybrid varieties and further suggests that increased seed size in hybrids does not cause greater pod-drop in these varieties.

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**Fig. 9** Average pod-retention resistance of 4 hybrid (Hyb - solid bars) and 4 open-pollinated (OP - pattern bars) canola varieties at Kelburn 2011 (black), Kelburn 2012 (white), and Carman 2012 (grey) determined using a force gauge. Within year and location, means with different letters are significantly different based on Fisher's protected LSD. Standard errors of the means and significance of single degree-freedom contrasts comparing the breeding types are indicated.

**Correlation between methods** - In order to evaluate objective 1 and 2 a correlation analysis was conducted among the different methods evaluated in this project. The methods investigated in this project estimate different components of yield losses in canola and therefore only correlations that address meaningful key questions are presented. Correlations were conducted on treatment averages of absolute and proportional yield loss (expressed as a percentage of yield) (Table 1). As there may have been isolated issues with predation and weather related seed loss from trays in 2011only data collected in 2012 were used for this correlation analysis. In a separate correlation analysis of the 2011 data alone, results were similar (data not shown) indicating that the impact of predation and weather related seed losses was relatively small.

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A comparison between total pre-harvest (catch tray total) and total pre- and post-harvest losses (vacuum method) was conducted and these two measurements correlated quite well when expressed as absolute or proportional values (Table 1). The correlation coefficients likely would have been greater had the catch trays been left in the plots until after direct-harvest as combine losses are not accounted for in the catch-tray measurements. One limitation of the catch tray method is that in narrow-row canola, removal and re-insertion of the trays in ripe stands is difficult to accomplish without causing some degree of harvest losses, particularly given the late stage at which these plots were direct-harvested. Therefore, it was decided to not re-insert the catch trays into the stand immediately before harvest. Without a measure of the portion of seed lost during the harvest process using the catch trays, results from the catch tray method and vacuum method were not directly comparable.

One of the more interesting questions addressed by this research was whether pod-drop and seed-shatter are related or independent events (Table 1). The correlation analysis confirmed observations of the catch tray results, which were that the relationship between these phenomena was weak based on the Pearson r correlation coefficients (absolute 0.14, proportional 0.28) and would be considered largely independent phenomena in these experiments. A Pearson r correlation coefficient of 0.28, for example, indicates that the only about 8% (0.28 x 0.28) of the variation in seed shatter is explained by pod drop and vice versa.

**Table 1.** Correlation analysis addressing key comparisons among the methods investigated. Pearson's r and the p-values for correlations of absolute and proportional measurements are indicated.

Correlation	Pearson's r	p-value
Pre-harvest (catch trays) vs. Total harvest loss (vacuum)		
absolute (kg ha-1)	0.81	< 0.0001
proportional (% of yield)	0.83	< 0.0001
Pod-drop (catch trays) vs. Seed-shatter (catch trays)		
absolute (kg ha <sup>-1</sup> )	0.14	0.2631
proportional (% of yield)	0.28	0.0230
Seed-shatter (catch trays) vs. Visual rating (seed-shatter)		
absolute (kg ha <sup>-1</sup> )	0.72	< 0.0001
proportional (% of yield)	0.67	< 0.0001
Average force (Pod retention resistance) vs. Pod-drop (catch trays) absolute (kg ha <sup>-1</sup> )		
raw force data (kgF)	-0.19	0.1247
adjusted force (kgF g*pod <sup>-1</sup> )	-0.54	< 0.0001
proportional (% of yield)		
raw force data (kgF)	-0.21	0.0960
adjusted force (kgF g*pod <sup>-1</sup> )	-0.48	< 0.0001
Crop yield vs. Total harvest losses (vacuum)		
absolute (kg ha <sup>-1</sup> )	0.41	8000.0
proportional (% of yield)	0.27	0.0282
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A key observation was that visual ratings provide reasonable estimates for absolute and proportional seed-shatter as determined by catch trays (Table 1). This result is encouraging in that the visual rating method is very fast and efficient. As canola yield, however, is difficult to estimate visually, one might expect that visual seed-shatter ratings provide a better estimate of absolute rather than proportional yield losses due to seed-shatter. The difference in the correlation coefficients between absolute and proportional seed-shatter yield losses indicate that this method was equally appropriate for estimating absolute and proportional seed losses due to seed-shatter.

The new method for determining pod-retention resistance examined in this project was compared to the pod-drop results from the catch trays. The raw pod-retention resistance data (kgF) did not correlate well to the amount of yield loss attributable to pod-drop determined using the catch tray method (absolute r = -0.19; proportional r = -0.21). Adjusting average pod retention resistance with pod weight, however, resulted in a dramatic improvement in the relationship between these data and absolute or proportional pod drop (absolute r = -0.54; proportional r = -0.48) among the 8 varieties that were compared. Overall, however, pod retention resistance was less variable among locations and years than actual pod-drop measurements (Figs. 6 and 9) which may have contributed to the correlation results. This method has the potential to be a tool for rapidly assessing pod drop in canola, however, refinement and validation across a more broad range of germplasm and different environments is required.

Interestingly, the correlations of absolute and proportional total pre- and post-harvest yield losses (vacuum method) (Table 1) and canola yield were similar to the results found on-farm (project 3.4.1) where absolute total harvest losses were positively correlated with canola yield and proportional total harvest losses were negatively correlated with canola yield indicating that proportional yield loss decreases with increasing canola yield.

**Digital images** - To evaluate the feasibility of using digital image capturing technology, images were collected from the greenhouse in 2010 and from field experiments during the 2011 and 2012 trials. Images were subjected to analysis based on separation of space and colour. Identification of pods against a soil background was possible in the greenhouse experiment with good success, while the identification of seeds on a clean soil background was more challenging due to the size and colour of seeds (Fig. 10).



Fig.10 Image analysis of pods based on separation of space and colour with Assess 2.0

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Analysis of images collected in the fields was more challenging. Sealed pods were not easily distinguishable from plant residue, other vegetation, or split-pods using this software package. Identification of canola seeds was even more challenging in the field due to difficulty in differentiating seeds from the soil background even after the crop residue had been removed. With the current software that did not have the capacity for shape and size recognition, it was not possible to adapt this software for rapid pod or seed recognition from digital images obtained from the field. Even pod-drop will be difficult to determine using digital image analysis as the pedicle remains attached to the pod and therefore leaves no recognizable mark/remnant on the rachis that could be easily detected using image analysis software.

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