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MANITOBA Canola Growers ASSOCIATION

Canola — Alberta Producers Commission — *Providing leadership for a vibrant, sustainable Alberta Canola Industry.*

FINAL REPORT

1.	Principal Investigator's Family Name: Gruber	Given Names: Margaret	Position: Research Scientist	Date: March 11, 2008
	Institution: Saskatoon Research Centre, AAFC		Department: Molecular Genetics	
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2.	Title of Research Project: Flea beetle and drought resistance in canola			
3.	Start Date (this report): April 1, 2005		Completion Date (this report): March 11, 2008	
4.	Original Project Objectives: To develop canola germplasm with protection against Flea beetles and drought by testing three strategies: 1) Develop new epidermis-specific GL3+ *Brassica napus* seed types into homozygous lines. Screen for transgene insertion lines with enhanced trichome density, resistance to flea beetles and drought, and good growth, and analyze the trichomes for metals or phenolics. 2) Isolate the ANGUSTIFOLIA and ZWICHEL branching genes from *Arabidopsis* and introduce them into GL3+ *Brassica napus* to stimulate trichome branching and promote additional flea beetle resistance. 3) Confirm flea beetle resistance in the ACC deaminase+ *Brassica napus* lines in field trials, and then re-combine suitable candidates with GL3+ lines and Lc+ lines .			

5. Abstract of Major achievements or discoveries during the 3 year grant:

Technologies have been developed to control the extensive feeding damage which occurs in Canola (*Brassica napus*) due to the crucifer flea beetle. New progeny from crosses between canola transformed with maize anthocyanin regulatory genes and 'HAIRY CANOLA' lines transformed with the Arabidopsis GL3 and GL1 genes continue to show improvements in growth while maintaining the dense trichome coverage shown in the initial generations of 'HAIRY CANOLA'. Promoter sub-fragments upstream from the GL3 gene were spliced together with a GUS reporter gene and have been over-expressed in *Arabidopsis*. Plants with these traits are now growing in the greenhouse and should help us to determine the promoter regions that control dense trichome coverage and normal growth. When complete, these experiments should enable us to design more effective strategies to express trichome genes in canola. A trichome-branching gene ANGUSTIFOLIA has been over-expressed in homozygous 'HAIRY CANOLA' line C67 in a *B. napus* cv. Westar background using two different selectable marker genes. The initial transformants are now flowering. T1 seed will be available in May 2008 to test whether this gene has the capacity to induce branched trichomes with greater coverage on the seedlings. We expect it to stimulate greater flea beetle resistance by decreasing the edible 'bare spots' still apparent to tiny flea beetles.

Laboratory insect choice and non-choice feeding bioassays and visual observances of flea beetle behaviour on 'HAIRY CANOLA' lines showed that flea beetles are still able to find small localized leaf areas bare of trichomes to feed upon, even though the trichome-enhanced lines are significantly less fed upon compared to the Westar control. Visual observations showed the beetles buried down between the trichomes deep enough to eat bare patches. They

either ignored the trichomes or, if they bit on a trichome and broke it off the leaf, immediately regurgitated it. Lines were not evaluated for metal, non-pigment phenolics, or tolerance to drought and cold (from the original objectives) since the homozygous trichome-bearing line C171, that has been the focus of our confined field trials and lab bioassays, was just recently recovered. In lab-choice feeding bioassays, the 48 hour rating consistently showed twice as many flea beetles on the Westar seedlings as compared to the 'HAIRY CANOLA' seedlings regardless of generation population or bioassay room temperature. The results of a 32°C bioassay showed extremely high feeding on all tissues of all seedlings except the stems of the 'HAIRY CANOLA'.

Additional achievements include the isolation of the *Arabidopsis* cDNA, AtMYB23, which has been introduced into *B. napus* cv. Westar. Once the plants have matured, the seed can be tested to determine whether trichomes were induced on the cotyledons.

In total, 20 Arabidopsis mutant lines with variation in trichome density and branching were recovered and are being evaluated to find novel genes that can increase trichome coverage, flea beetle resistance, and potentially, drought tolerance.

6. *Background:*

Approximately $200M damage occurs annually to the Canadian canola crop from the crucifer flea beetle in spite of $40M in annual chemical application costs to control these pests. Crop losses due to drought in Canada range from $300M to $1B annually. At present, there is no useful canola germplasm available to develop flea beetle resistant canola, and deregulation has restricted the use of several major insecticides. With the diversification of canola into both food and biodiesel streams, a huge increase in the supply of canola and associated new Brassica oilseed crops will be needed to maintain canola as Canada's (and one of North America's) top oilseed crop. Canola with resistance to flea beetles is urgently required to reduce productions costs and to avoid the risks to health inherent with pesticide use.

Plant breeders and entomologists have struggled unsuccessfully for years to develop canola germplasm with effective resistance to flea beetles. In the initial phase of this project, we introduced two *Arabidopsis* trichome regulatory genes into *Brassica napus* and stimulated dense coverage of ~1000 trichomes per cm^2 on seedling leaves. In the 2nd phase, detailed in this report, we screened for homozygous lines and improved the growth of these plants by crossing them with other lines that have normal growth and hairy stems. We also isolated an *Arabidopsis* trichome-branching gene and a newly discovered trichome-stimulating regulatory gene in an effort to increase the coverage of trichomes on the seedling plant surface of *Brassica napus* and to expand the affected tissues to include cotyledons. These latter experiments were initiated to cover some of the bare spots that tiny flea beetles could find and damage on our 'HAIRY CANOLA' lines. Finally, we conducted research into factors that control trichome gene expression, and have determined that trichomes genes should be expressed in cell layers below the epidermis.

Gruber, M., Wang, S., Holowachuk, J., Ethier, S., Soroka, J., Bonham-Smith, P., and Lloyd, A. 2006*. "HAIRY CANOLA" – Arabidopsis GL3 induces a dense covering of trichomes on Brassica napus seedlings. Plant Molecular Biology. 60: 679-698.*

7. Project Objectives:

Original:

To develop canola germplasm with protection against flea beetles and drought by testing three strategies:

1) Develop new epidermis-specific $GL3^+$ *Brassica napus* seed types into homozygous lines. Screen for transgene insertion lines with enhanced trichome density, resistance to flea beetles and drought, and good growth, and analyze the trichomes for metals or phenolics.

2) Isolate the ANGUSTIFOLIA and ZWICHEL branching genes from *Arabidopsis* and introduce them into GL3+ *Brassica napus* to stimulate trichome branching and promote additional flea beetle resistance.

3) Confirm flea beetle resistance in the ACC deaminase$^+$ *Brassica napus* lines in field trials, and then re-combine suitable candidates with $GL3^+$ lines and Lc^+ lines.

Expanded:

The original objectives were expanded in response to data collected during the project and due to the financing of a grant-supported molecular biology trainee on AAFC A-base funding for 4 months. This enabled CCC-WGRF funds to support undergraduate students at Queen's University and SRC to screen 10,000 Arabidopsis mutants for new genetic determinants of trichome density and structure.

Revised Objectives:

1. Evaluate *B. napus* seed lines from diallelic crosses between *GL3*$^+$ lines, *GL1*$^+$ lines and *Lc*$^+$ lines for new variation in resistance to flea beetles, cold temperatures and for improved growth.

2. Isolate and characterize trichome-branching and trichome regulatory mutations and genes from *Arabidopsis*.

3. Determine trichome gene expression determinants using plants transformed with *GL3*$^+$ promoter deletion derivatives fused to the *GUS* reporter gene.

4. Initiate a study to characterize trichome regulation in the Brassicas by characterizing Brassica homologues of several trichome regulatory genes in a trichome-rich relative of *Brassica napus*.

8. Experimental Method:

1. Advancing 'HAIRY CANOLA' lines:

Reciprocal crosses were conducted between GL1$^+$/GL3$^+$ lines, B-Peru$^+$ lines and Lc$^+$ lines of transgenic Brassica napus cv. Westar by removing the stamens of recipient plants and 'painting' the sigma with pollen from donor plants. Pollination bags were placed on the fertilized flowers and plants grown to maturity. Transgene stability and expression were analzed by PCR. Progeny plants grown in a greenhouse were phenotyped for degree of seedling anthocyanin coloration, trichome density and coverage, size and developmental rate, and seed yield. Seed increases were done on F1 plants with unique phenotypes but additional seed increases need to be done to improve plant vigour. A heterozygous 'HAIRY CANOLA' seed line was sown in replicated CFIA-approved confined field trial plots at the Saskatoon and Lethbridge farms of AAFC. Growth characteristics and flea beetle damage to cotyledons and true leaves were measured under field conditions at the seedling and mid-vegetative stages to provide a profile under field conditions. Flea beetle damage on 'HAIRY CANOLA' seedlings was tested along with a B. napus cv. Westar control in laboratory bioassays. Uniform seedlings were selected from a heterozygous seed line and randomly arranged in a replicated 4 x 8 design. Pre-feeding behaviours of flea beetles were recorded on 'HAIRY', Lc$^+$, and B. napus cv. Westar canola, using a dissecting microscope and integrated software package 'The Observer' (Noldus Information Technology Co.). In total, 8 pre-feeding behaviours were analyzed according to Henderson et al., 2004. A multivariate analysis of variance (MANOVA) was used to determine differences between durations of pre-feeding behaviours using Least Means Squares (SAS Institute, 2001) and a paired comparison t-test was used to analyze differences between on-and-off pre-feeding time within each genotype (SAS Institute, 2002). 'HAIRY CANOLA' lines were propagated using single seed descent to search for homozygous lines.

2. Understanding trichome architecture and organ-specificity in 'HAIRY CANOLA' with Arabidopsis trichome regulatory genes:

Full length cDNAs for the Arabidopsis trichome-branching genes ANGUSTIFOLIA, ZWICHEL, and AtMYB23 were amplified using gene-specific primers and PCR. ANGUSTIFOLIA and AtMYB23 were transformed into *B. napus* cv. Westar (Sun Lee, 1996 Ph.D.) or into C67 (DeBlock et al., 1989), one of the homozygous 'HAIRY CANOLA' lines. Putative transformants were transferred into a soil-less potting mixture, and grown in a greenhouse supplemented with high pressure sodium lamps.

3. Understanding factors that control cell layer-specific trichome gene expression:

A 2.5 kb fragment upstream from the Arabidopsis GL3 trichome regulatory gene was isolated and deletion derivatives were cloned covering different regions of the promoter sequence. These derivatives were ligated to a GUS histochemical staining reporter gene and introduced into *Arabidopsis thaliana* cv. Columbia (Clough and Bent, 1998). In other experiments involving an Arabidopsis 35S-enhancer T-DNA mutant line population (developed previously at SRC by Dr. I. Parkin), a 10,000 line sub-set of 40,000 was screened visually for trichome density and trichome abnormalities. Mutated seeds were sterilized in weak bleach and grown on MS tissue culture media on large format petri dishes. Seedling trichomes were observed under a light microscope. Selected lines with unique trichome phenotypes were grown to maturity and seed was collected. Plasmid rescue and TAIL-PCR were used to analyze a limited number of selected lines to determine genes affected by T-DNA insertion.

4. Initiating a study to characterize trichome regulation in the Brassicas:

Trichome-rich Brassica germplasm was obtained from Plant Gene Resources Canada and Carolina Seeds (Carolina Biological Supply Company, Burlington, N.C.). Seeds were potted and grown as above in a greenhouse. Seedlings and other developmental stages were evaluated for growth, trichome density and coverage, and flowering time. Photographs were taken during seedling development.

9. Results and Discussion:

Advancing 'HAIRY CANOLA' lines:
Progeny developed from cross-pollinations between GL1+ B. napus lines and GL3+ B. napus lines were advanced in a greenhouse and tested in CFIA-approved confined field trials in Lethbridge and Saskatoon. These experiments showed that the presence of this construct triggered the development of dense trichome coverage on 'HAIRY CANOLA' seedling leaves (Figure 1). These hairy leaves showed significant resistance to flea beetle feeding in field trials held in Saskatoon and Lethbridge (Figure 2 & 3). In addition, at early stages (6 days) of cotyledon growth, the presence of the GL3 gene deterred flea beetles from feeding in the Saskatoon trial, but at the 14 day rating the feeding deterrency dissipated (Figure 2). This early resistance was likely due to a change in the chemical composition of the hairless cotyledons. We did not see this same early deterrence in the Lethbridge field trial (Figure 3). Confined field trials have not taken these more advanced 'HAIRY CANOLA' plants to seed yield yet because of the only recent development of the C171 homozygous seed line.

Two 'HAIRY CANOLA' homozygous seed lines have now been developed: C67 seedlings have early true leaves and stems which are very hairy, C171 seedlings have very hairy true leaves and stems as well as trichomes below the apical meristem. Both of these homozygous seed lines have smaller cotyledons and true leaves in the very early seedling stages as compared to parental Westar, as well as a smaller stem diameter (Figure 1). However, these lines develop slightly faster up to the 3rd true leaf stage under greenhouse conditions compared to the Westar parental control (Figure 1), and mature only 1.5 to 2 weeks later than the parental control line. Seed yield of these 2 homozygous lines under greenhouse conditions is similar to Westar seed yield.

Non-choice lab bioassays with B. napus cv Westar and 'HAIRY CANOLA' showed that spring flea beetles will move on and off a trichome-enhanced leaf surface twice as frequently as on a control leaf with low trichome numbers, where they stay on the surface and feed. More than 50% of the total flea beetles introduced to the control leaf tissue feed, while only 25% of flea beetles feed on trichome-enhanced tissue. Flea beetles seem to be confused by the trichome-enhanced tissue and increase their feeding time two-fold compared with feeding on the control tissue. They have difficulty maneuvering on the trichome-enhanced leaves and cannot easily contact the leaf surface with antennae and mouthparts or tap with their tarsae. This reduces their ability to make a decision on host plant palatability. This behavioral response is not always seen when flea beetles are given a choice of host plants in close proximity.

In choice lab bioassays at 21°C, flea beetles were moderately active but they had a different response to 'hairy' canola plants compared with non-choice bioassays. When the number of flea beetles present on individual seedlings was recorded at 48 hours, consistently more flea beetles were on the control seed line (Figure 4). Feeding damage at 48 hours was twice as high hairless control cotyledons for both the spring and fall insect populations compared with the hairless transgenic cotyledons (Figure 4). Feeding damage on the first two 'hairy' true leaves was similar for the control and 'hairy' transgenic plants regardless of the difference in trichome density (Figure 4).

In choice lab bioassays at 32°C the 'hairy' stems of the transgenic plants had no feeding damage, while the hairless stems of neighboring control plants were heavily fed upon (Figure 4). This could be due, in part, to the disruption of the anthocyanin pathway in the green transgenic plants as well as possible disruptions in other metabolic pathways caused by transgene expression from a constitutive 35S promoter. At this higher temperature, the cotyledons and true leaves of both seed lines sustained equal feeding damage and left a skeleton of trichomes on the 'hairy' leaves (Figure 4). The insects appeared to feed on localized hairless areas on the 'hairy' leaf tissue.
Additional cross-pollinations between Lc+, GL1+/GL3+, and B-Peru+ transgenic lines of B. napus are now producing more advanced lines with dense seedling trichome coverage and improvements in growth compared with the original 'HAIRY CANOLA' lines (Figure 1). These new 'very hairy' F1 progeny have more robust green stems, dark purple coloration on the underside of the cotyledons, and larger phenotypes at the seedling stage than previously seen in less advanced lines. Most of the lines with the enhanced trichome densities are still segregating.

Testing trichome branching genes for efficacy in Brassica napus:
The full-length cDNA for the Arabidopsis trichome-branching gene ANGUSTIFOLIA (AN) has been cloned, sequenced and introduced into the earlier homozygous 'HAIRY CANOLA' seedline C67 as well as B. napus cv. Westar. Greenhouse-grown transformants have been confirmed to have the AN transgene by PCR. Trichome branching data should be available on T1 progeny in May 2008. The full-length Arabidopsis trichome-branching cDNA ZWICHEL has been cloned, but is still proving difficult to sub-clone into a plant binary vector because of its large size. If the AN gene functions correctly in Westar, this may prove to be a useful method to increase trichome complexity enough to cover more leaf areas and prevent flea beetles from burrowing down and feeding on bare areas.

Testing a cotyledon trichome induction gene in Brassica napus:
An Arabidopsis cDNA AtMYB23, which induces trichomes on Arabidopsis cotyledons, has been cloned, sequenced, ligated into binary constructs containing a kanamycin-resistance selectable marker gene and transformed into B. napus cv. Westar. These putative transformants are growing in the greenhouse at SRC in preparation for the evaluation of cotyledon trichomes on T1 seedlings in June 2008.

Determining factors affecting the expression pattern of trichome genes:
An epidermal-specific promoter from the Arabidopsis CUT1 gene was linked to the GL3 gene and introduced into Brassica napus cv. Westar. The resulting plants had normal growth characteristics, but did not show any increase in trichomes. Overlapping sub-fragments covering the GL3 promoter were cloned into a binary vector driving a GUS reporter gene. Arabidopsis T0 plants over-expressing these sub-fragments or the full-length promoter are now growing in the greenhouse in preparation for GUS histochemical staining. When complete in 2008, these experiments should enable us to determine the correct cell layer(s) in which to express the GL3 gene without compromising plant growth. At the present time, we anticipate that the sub-epidermal layer is more important for GL3 expression than the epidermis.

In a collaboration between Dr. Isobel Parkin. Dr. Gruber of SRC, and Dr. Sharon Regan, Dept. of Biology, Queen's University, Kingston, a total of 20 enhancer mutant lines of Arabidopsis were recovered with variation in seedling trichome density and structure from 10,000 of the Arabidopsis enhancer lines. The mutants are tools for identifying novel Arabidopsis trichome-branching genes and for finding new determinants important for trichome development (Figure 5). Several of the mutant lines were characterized using molecular techniques by an undergraduate thesis student Yun Yun Wu at Queen's University, but additional analysis is required to determine if these are unique mutants or alleles of known genes.

Initiation of a study on trichome molecular biology in the Brassicas:
Evaluation of the growth of trichome-rich Brassica villosa indicated a very strong barrier to flowering. B. villosa plants have been subjected to drought and nutrient deficiency conditions to try and overcome this barrier, but to date these plants have not flowered and are still under evaluation. Contacts were made with Graham King (Wellsborne University, UK), who agreed to provide fast flowering hybrids of B. villosa and B. rapa for a study on non-GMO trichome enhancement using interspecific crossing. Rapidly-growing 'hairy' B. rapa FAST PLANTS from the University of Wisconsin collection have also been evaluated and appear to have increased trichome coverage in some of the plants. A graduate student, Ushan Alahakoon, was accepted into the Ph.D. program in the Biology Dept at the University of Saskatchewan and has initiated thesis work on the molecular biology of trichomes in the Brassica triangle of U. This approach should produce valuable information to assist us in developing flea beetle resistant canola based on dense trichome coverage.

Summary:

The experiments from the past 3 years of research on 'HAIRY CANOLA' and trichome regulatory genes are producing the world's first germplasm with strong potential to develop into flea beetle resistant canola breeding germplasm but require several more years in order to understand how to increase trichome coverage in *B. napus* without compromising growth The plants were developed using GMO strategies and should be widely accepted in Canada for their pesticide reduction value. Equivalent germplasm developed using non-GMO methods would likely be even more preferred for international canola markets.

10. Publications/Communication of Results:

Gruber, M., Wang, S., Holowachuk, J., Ethier, S., Soroka, J., Bonham-Smith, P., and Lloyd, A. 2006. "HAIRY CANOLA" – Arabidopsis *GL3* induces a dense covering of trichomes on *Brassica napus* seedlings. Plant Molecular Biology. 60: 679-698.

Invited Conference Presentation: Gruber, M., Wu, L., Holowachuk, J., Yu, M., Hegedus, D., Soroka, J., Xu, N., and Sharpe, A. 2007. 'THE DINNER PARTY': Brassica and Arabidopsis meet the crucifer flea beetle. Abstract and Invited Oral presentation by M. Gruber. Plant Animal Genome Conference XV. Jan 13-17. Brassicas Workshop. pg 9.

AAFC Internal Meeting Presentation: M. Gruber, D. Cui, L. Wu, B. Coulman, I. Parkin. 2007. 'ALL DRESSED UP WITH NO PLACE TO GO": Novel coat patterns and new "hairdos" in a population of activation-tagged Arabidopsis lines. Oral presentation by M. Gruber. AAFC Canadian Crop Genomics Initiative Annual Meeting. Penticton, BC.

Conference Presentation: M. Gruber, J. Holowachuk, L. Wu, M. Yu, J. Soroka, D. Hegedus. 2007. 'THE DINNER PARTY': *Brassica* and *Arabidopsis* meet the crucifer flea beetle. Abstract and Poster presentation by M. Gruber: The 12th Int'l Rapeseed Congress. "Sustainable development in cruciferous oilseed crops production". Wuhan, China, March 26-30, 2007. BP-1-31.

Conference Presentation: Holowachuk, J.M., Gruber, M. and Soroka J. 2007. Behaviour of flea beetles on hairy *Brassica napus* expressing trichome genes from *Arabidopsis*. Abstract and poster presentation by J. Holowachuk. "Insects: Microscale Subjects for Megascale Research. Entomological Society of Canada Annual Meeting. Saskatoon, SK., Sept. 29-Oct. 3, 2007. Poster#16. pp. 45.

Conference Presentation: Soroka, S., Gruber, M., Holowachuk, J. and Grenkow, L. 2006. "Hairy Canola" meets flea beetles: behavior and damage. Abstract and Oral Presentation. 56th Annual Meeting. Entomological Society of Canada. Montreal, PQ. Nov. 18-22.

Invited Oral Presentation: Gruber, M. 2006. Modifying plant secondary metabolites and plant morphology for crop quality and protection. Inner Mongolia Agricultural University, Hohhot, China. Oct. 23.

Invited Oral Presentation: Gruber, J. 2006. Modifying plant secondary metabolites and plant morphology for crop quality and protection. Forage and Grasslands Institute, Chinese Academy of Agricultural Sciences. Hohhot, China. Oct. 23.

Oral presentation: Gruber, M., Soroka, J., Holowachuk, J., Grenkow, L., Yu, M., Parkin, I. and Regan, S. 2006. Flea beetle and drought resistance in canola. Annual meeting. Saskatchewan Canola Development Commission, Canola Council of Canada, Agriculture and Agri-Food Canada. Saskatoon, SK, Dec. 16.

Media interview: 'Hairy Canola' with Kevin Hersch. Sponsored by Saskatchewan Canola Development Commission for radio spot for Saskatchewan farmers on Aug. 18, 2007. Aired on CJWW Saskatoon, CKRM Regina, CKSW Swift Current and GX94 Yorkton.

Media interview: 'Hairy Canola' with Bob Simpson on April 10, 2006. Aired on Prairie Farm Report / Farm Gate. CTV media.

11. Impact:

'HAIRY CANOLA' lines developed through this proposal have the potential after further advancement to significantly reduce crop input costs and provide consistent protection without the need for pesticide application and exposure. They may well be more tolerant to cold and wind desiccation due to the insulating effect of plant hairs noted on desert plants (Ehleringre, 1984). 'HAIRY CANOLA' lines will be based on multi-gene traits which confound insect behaviour. This type of resistance will be more durable compared with antibiotic mechanisms based on plant insecticidal chemicals or proteins that have been shown to break down quickly (Van Mellaert et al. 1989). When developed into varieties, the seed will be purchased preferentially by canola growers. Pioneering these lines will create new wealth for Canadian seed companies and farmers. It will protect farmers from health-related pesticide problems, sustain and improve the safe supply of their canola, and improve soil and run-off in agricultural lands, by eliminating the need for pesticide application. Increased seed yields/acre (due to reduced crop damage) will result in increased farm and seed company profits and lead to an expansion of the Canadian canola acreage and a greater share of the expanding national and international oilseed and oil crush markets. The technology will also be useful for Ethiopian mustard, which is being bred at SRC for biodiesel applications.

12. Collaborating Scientists and Roles:

Dr. Margaret Gruber (Brassica/Arabidopsis Genomics, Trichomes – lead investigator), Saskatoon Research Centre, AAFC, Saskatoon, SK
Dr. Julie Soroka (Entomology), Saskatoon Research Centre, AAFC, Saskatoon, SK
Dr. Isobel Parkin (Brassica/Arabidopsis Genomics), Saskatoon Research Centre, AAFC, Saskatoon, SK
Dr. Sharon Regan (Genomics, Trichomes), Department of Biology, Queen's University, Kingston, ON

13. Project Staffing and Training:

Saskatoon Research Centre:
Jennifer Holowachuk – acquired skills in basic and advanced plant molecular biology theory and techniques and responsible for conducting CFIA-approved confined field trials.
Angela Schindelka – acquired basic lab and plant maintenance techniques, and monitoring of CFIA-approved confined field trials.

Queen's University:
Yun-Yun Wu - acquired basic plant microscopy techniques and rapid techniques for screening Arabidopsis seedlings.
Dave Lin and Jordan Chang - two additional summer students trained in rapid techniques for screening Arabidopsis seedlings.

14. Literature Cited:

De Block, M., De Brouwer, D., Tenning, P. 1989. *Agrobacterium tumefaciens* and the expression of the bar and neo Genes in the transgenic plants. Plant Physiol. 91: 694-701.

Ehleringre, J. 1984. Ecology and ecophysiology of leaf pubescence in North American desert plants. In: Rodriguez, XE., Healey, PL. and Mehta, I. (eds.). Biology and chemistry of plant trichomes. Plenum Press, New York, pp. 113-132.

Van Mellaert, H., Hofte, H., Reynaerts, A. and Vaeck, M. 1989. Insect-resistant transgenic plants expressing Bacillus thuringiensis genes. Plant-Microbe Interactions 3: 3-10.

15. Submitted by:

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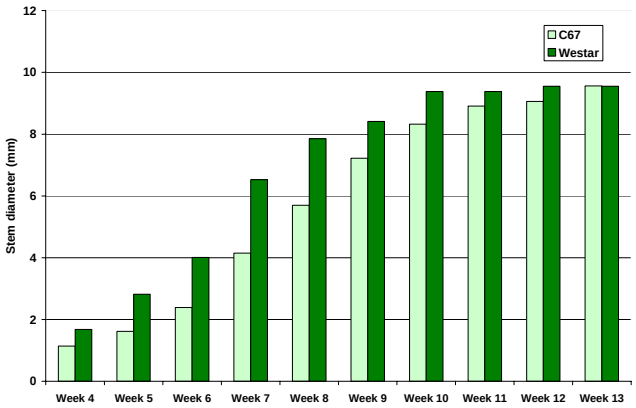
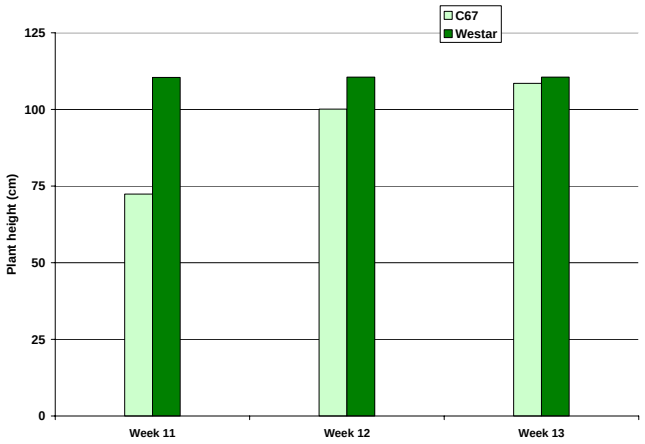
Figure 1. Advanced lines of 'HAIRY CANOLA' growing in the greenhouse



Average stem diameter (mm) of C67 and Westar. Initially, the GL1^{+}/GL3^{+} cross has a weaker stem but improves as growth progresses.

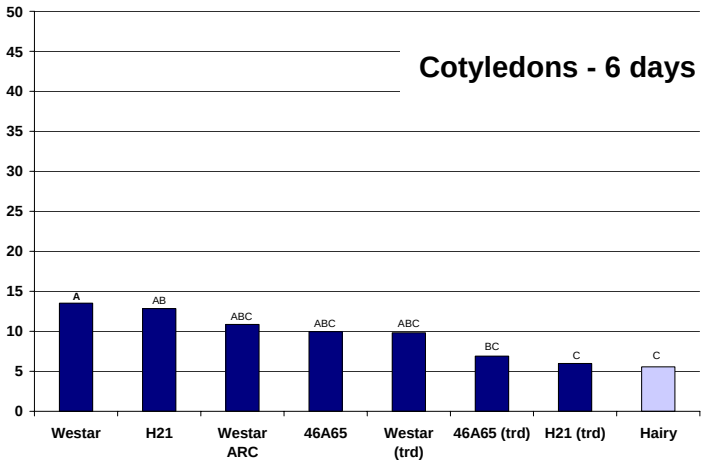


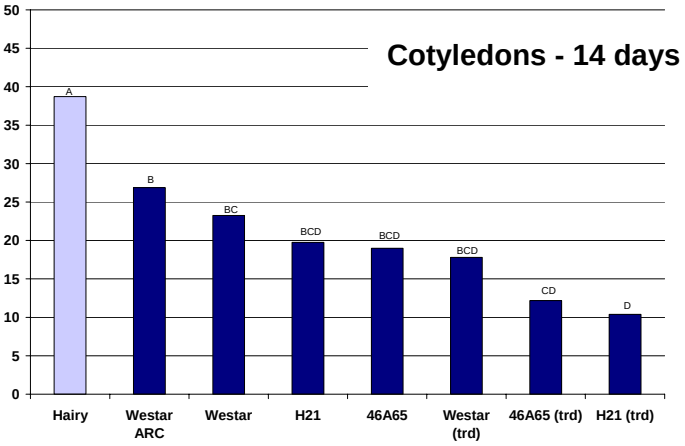
Average plant height (cm) of C67 and Westar. The maximum plant height for the GL1^{+}/GL3^{+} cross is delayed by 2 weeks.

Growth comparison of Westar (right) and C171 (far right) in greenhouse conditions. Note: C171 has it 3rd true leaf emerging while Westar has only its 2nd true leaf emerging. In comparison, C171 has smaller cotyledons and true leaves and lacks anthocyanin coloration in the stem and leaf petioles.

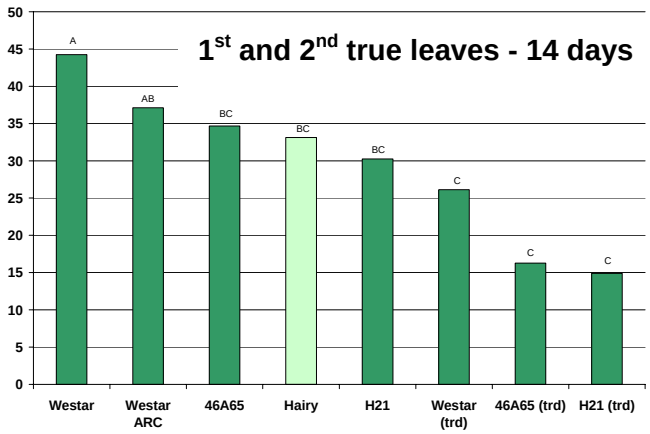
Left: Unique phenotype of a seedling of C171 (GL1^{+}/GL3^{+}) x B-Peru^{+} cross. Note dark purple coloration on the underside of the cotyledons and 'very hairy' stem and true leaves.
Below: Stunted phenotype of the same seedling nearing seed set.

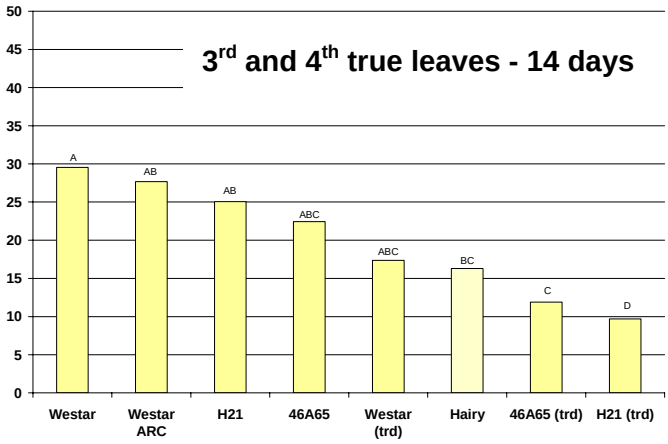
Figure 2. Saskatoon 2006 field trial testing C171 (GL1$^+$/GL3$^+$ cross) 'HAIRY CANOLA' under moderate flea beetle feeding pressure.





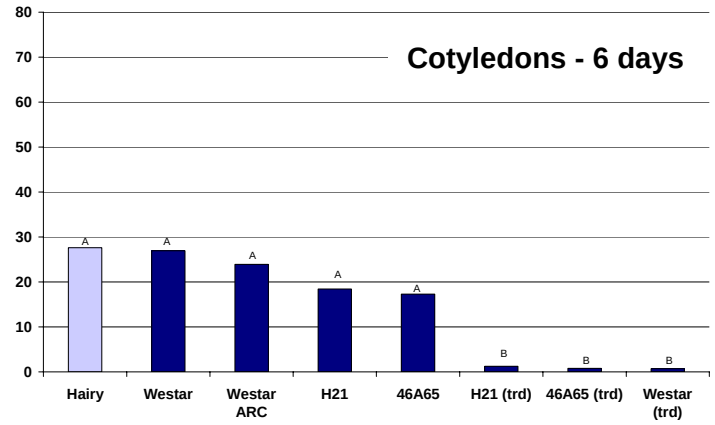
% Feeding damage ratings in Saskatoon at 6 and 14 days after seedling emergence on GL1$^+$/GL3$^+$ '*HAIRY CANOLA*' cotyledons and first 4 true leaves compared with untreated Westar parental control line and several other lines(H21, 46A65, Westar ARC). Treated (trd) seed lines were protected with a seed applied pesticide. Differences in letters at top of bar graph indicate statistical significance.

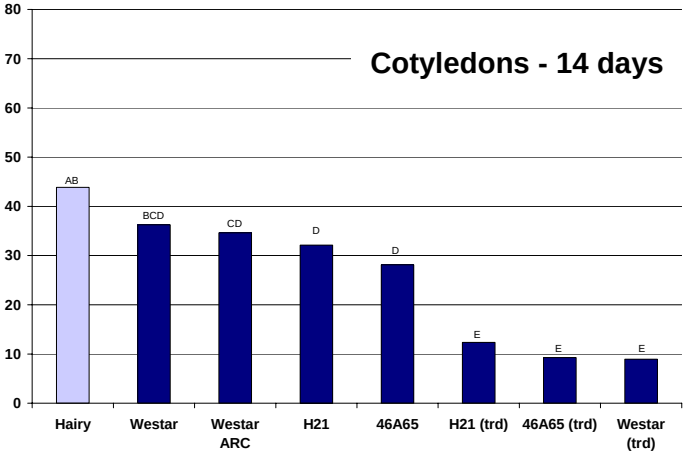




Lines in the 2006 Saskatoon confined field trial displayed even and high germination rates. 'HAIRY CANOLA' leaves with dense trichome coverage were consistently resistant to flea beetles. Smooth hairless cotyledons of 'HAIRY CANOLA' were resistant immediately after emergence, but became highly susceptible when hairy true leaves appeared.

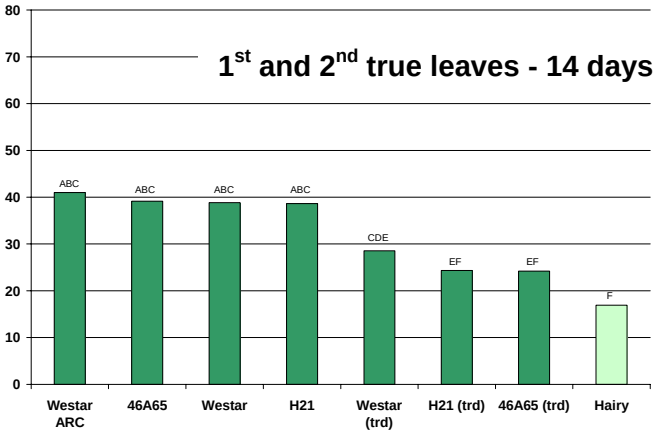
Figure 3. Lethbridge 2006 field trial testing C171 (GL1^{+}/GL3^{+} cross) 'HAIRY CANOLA' under extreme flea beetle feeding pressure.





2006 Lethbridge field trial with strong winds, cold temperatures and rain. Lines sustained extremely variable germination rates due to field conditions not seed quality.

The 'HAIRY CANOLA' hairless cotyledons showed no resistance when faced with extreme flea beetle feeding pressure at this trial site. True leaves with dense trichome coverage were less fed upon than controls.



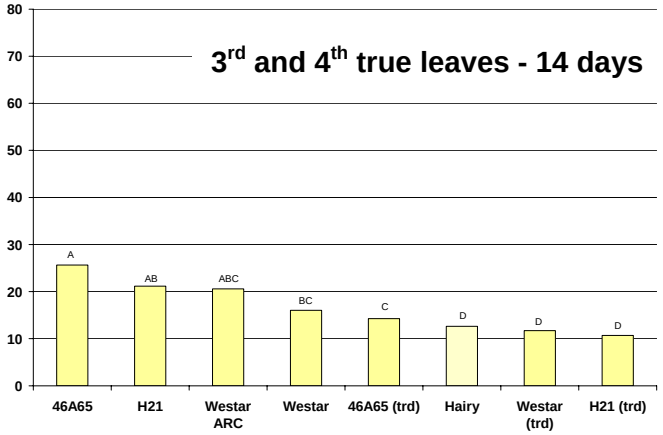
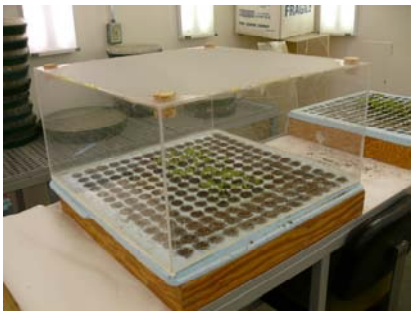


Figure 4. Laboratory feeding and behavior bioassays on C171 (*GL1*$^{+}$/GL3^{+}) 'HAIRY CANOLA'

Flea beetles were agitated on 'HAIRY CANOLA' leaves and could not settle down into their normal feeding pattern. Instead, they tried to feed through the trichome mat by standing on their heads. Flea beetles were initially curious and spent more time on trichome-bearing surfaces than on smooth surfaces. However, at the end of the bioassays (48 hours), there were consistently twice as many flea beetles counted on the Westar control plants.

Average # of Flea beetles / plant after 48 hours	
Westar	3.8
Hairy canola	1.9

At 21°C, after 48 hours, the tissue area consumed by flea beetles was much lower for cotyledons, leaves, and stems of the 'HAIRY CANOLA' seedlings, compared with the Westar control seedlings. This data suggests that the GL3 gene has altered the biochemistry of the stems and hairless cotyledons and provided chemical-based protection for the plants at that growth stage, in addition to the physical barrier of dense trichome coverage on seedling leaves.



% Tissue area consumed after 48 hours		
	Cotyledons	1st and 2nd true leaves
Westar	15.0	30.1
Hairy canola	6.2	23.1

Flea beetle choice lab bioassay at 32°C. Far left: Highly fed upon hairless stem of Westar seedling Left: No feeding at all on 'hairy' stem of 'HAIRY CANOLA' seedling. Note skeleton of trichomes left behind on true leaves.

Figure 5. Sampling of trichome variation seen in 10,000 lines of an *Arabidopsis* 35S-enhancer mutant population.