Marker-Assisted Breeding for Fusarium Wilt Resistance in Canola

CARP Project Number 2005-10

Alberta Research Council and Agriculture, Vegreville, AB

and

Agri-Food Canada, Saskatoon, SK

01 June, 2009

Table of Contents

List of Figures

List of Tables

Acknowledgements

Funding for this project was provided by the Alberta Agricultural Research Institute, the Agriculture and Food Council, and the Canola Council of Canada
We are grateful for the technical assistance of Wendi Dmytriw, Sharla Eldridge, and Sarah Kuzmicz.

Marker-Assisted Breeding for Fusarium Wilt Resistance in Canola

Ralph Lange - Alberta Research Council, Vegreville, AB
Sarah Kuzmicz – Agriculture and Agri-Food Canada, Saskatoon, SK
S. Roger Rimmer – Agriculture and Agri-Food Canada, Saskatoon, SK

Abstract

Fusarium wilt (FW) of canola has caused substantial losses since it was identified in 1999. Genetic resistance is the most cost-effective and probably the only effective method of controlling FW. Plant breeders could theoretically eliminate susceptible genotypes from their current and future simply by screening out susceptibility to FW early in crossing programs. However, this approach precludes introgressing desirable traits from or into FW-susceptible genotypes. Knowledge of the mode of inheritance would improve the ability of plant breeders to manipulate the FW-resistance trait. Genetic markers can be used to differentiate plants that carry a desired trait (such as FW resistance) from those that do not. Markers are selected that are in close physical proximity to the gene(s) so that they will co-inherit with the desired trait as each generation of plants are produced. This genetic linkage means that breeders can directly analyse the DNA of very young plants and need not wait for the plant to mature so they can be tested: an ability that is particularly important with FW as symptoms in field trials only become visible when infected plants approach physiological maturity, or in controlled-environment tests. The objectives of this project were to identify and characterize the mode(s) of inheritance of resistance(s) to *F. oxysporum* in *Brassica napus*, and to identify molecular markers linked to resistance traits to FW utilizing *B. napus* populations segregating for resistance and susceptibility to the pathogen. Analysis of *F. oxysporum – B. napus* F_1 and F_3 populations generated from crosses between FW-susceptible (Canterra 1604) and resistant (SP Banner and DH12075) parents indicated that FW-resistance is conferred by a single dominant gene. The FW-resistance locus, named FusR1, was associated with microsatellite markers sR536 and sR634OI, located on the N3 linkage group (A-genome) on the Agriculture and Agri-Food Canada *B. napus* consensus map.

Introduction and project objectives

Fusarium wilt (FW), caused by *Fusarium oxysporum* (Schlecht.) Snyder et Hansen, causes severe yield losses of canola (Lange et al, 2007). In susceptible cultivars of *Brassica napus* L., symptoms progress from a bright-yellow, often localized chlorosis, to necrosis of leaves, stems and siliques, and finally to premature desiccation of siliques and malformation of seeds. FW has occurred as problem of commercial crops in Russia (Nikonorenkov V.A. et al, 1996;Portenko, 1998), western Canada (Lange et al, 2000; Pearse et al, 2000; Pearse et al, 2001; McLaren D. L. & Platford, 2001; Benard et al, 2001; Benard et al, 2002; Pearse et al, 2003; McLaren D. L. et al, 2003; Benard et al, 2003; Pearse et al, 2004; Lange & Franke, 2004; Pearse et al, 2005; McLaren D. L. et al,

2005; McLaren D. L. et al, 2005; Dmytriw &Lange, 2005), and Argentina (Gaetan, 2005a;Gaetan, 2005b;Gaetan, 2005c).

Yield losses due to FW can be eliminated through the use of resistant cultivars. In comparisons of susceptible and resistant *B. napus* genotypes in field trials at nine locations in western Canada, mean yield of the least-affected cultivar was 15.9% higher than the most severely affected cultivar; yield improvement due to disease resistance increased to 75.2% when the least- and most severely-affected cultivars were compared at the site with the greatest disease pressure (Lange et al, 2007). FW-susceptible cultivars or breeding lines can be identified using growth chamber and field screening techniques (Lange et al, 2007). Use of these techniques has allowed plant breeders to identify susceptible germplasm in breeding programs.

Plant breeders could theoretically eliminate susceptible genotypes from their current and future simply by screening out susceptibility to FW early in crossing programs. However, this approach precludes introgressing desirable traits from or into FW-susceptible genotypes. Knowledge of the mode of inheritance would improve the ability of plant breeders to manipulate the FW-resistance trait.

The mode of inheritance of FW resistance in *B. napus* is unknown, but has been described for Fusarium yellows of *B. oleracea* crops, which is also caused by *F. oxysporum*. It has been known since the early part of the last century that resistance to *F. oxysporum* in *Brassica oleracea* L. can be conditioned by a single, dominant gene (Type A) or can be under polygenic control (Type B) (Blank, 1937;Walker, 1926;Walker, 1930). Type A resistance in *B. napus* would simplify introgression of FW resistance into susceptible genotypes, particularly if classical selection and introgression techniques are used.

Ideally, FW resistance would be linked to DNA-based genetic markers. Marker-assisted selection would allow breeders to directly infer the genotype of very young plants, and avoid time delays caused by phenotype testing, and simultaneously avoid confounding effects of genotype × environment interactions. Resistance to FW has not been associated to molecular markers in *B. napus*, although this has been done for vascular Fusarium wilts of other crop species. Examples include bean (Fall et al, 2001), chickpea (Halila et al, 2009), lentils (Hamwieh et al, 2005) eggplant (Mutlu et al, 2008), tomato (Lim et al, 2006), and melon (Oumouloud et al, 2008).

We set out to infer the mode of inheritance of the FW-resistant phenotype, determine the number of genes associated with resistance and identify microsatellite markers that are linked with those genes.

Materials and methods

Plant materials and segregating populations

Figure 1 diagrams the crosses made to generate *B. napus* populations segregating for Fusarium wilt resistance/susceptibility. The cultivars SP Banner and DH12075 were

reciprocally crossed as resistant parents with Canterra 1604 as the susceptible parent. All cultivars are doubled haploid-derived, open-pollinated genotypes. The susceptibility or resistance of the parental plants to FW was determined before the initial crosses were performed, using cuttings taken from the parents before inoculation. The F_1 progeny were self-pollinated and grown to maturity to produce four F_2 plants (two for each original susceptible × resistant cross). Self-pollination of the F_2 plants resulted in between 86 and 99 F_3 individuals for each original cross. F_3 plants were then self-pollinated to produce seed for phenotype testing.

Phenotype testing

F. oxysporum – B. napus interation phenotypes were determined in controlled environment tests. No phenotyping trials were conducted as field tests because controlled environment trials were found to accurately reflect resistance phenotype without the confounding effects of environmental conditions (data not shown). Twenty seeds of each genotype were sown in 20" × 12" × 6" steel food service trays (Russell Food Equipment, Edmonton, Alberta) filled with 10L of industrial quartz sand (SIL Industrial Minerals, Edmonton, Alberta) trays filled with silica sand. The plants were fertilized with SI nutrient solution (500ml per L water), prepared by adding 10ml each of solutions A, B, C, and D to 20L of reverse osmosis-purified water; Solution A consisted of 472.3 g/L $Ca(NO_3)_2 \cdot 4H_2O$, 153.84 g/L $Mg(NO_3)_2 \cdot 6H_2O$, and 48.026 g/L NH_4NO_3, Solution B consisted of 34.836 g/L K_2HPO_4, 34.852 g/L K_2SO_4, and 80.886 g/L KNO_3, Solution C contained 0.792 g/L $MnCl_2 \cdot 4H_2O$, 0.742 g/L H_3BO_3, 0.288 g/L $ZnSO_4 \cdot 7H_2O$, 0.075 g/L $CuSO_4 \cdot 5H_2O$, and 0.048 g/L $Na_2MoO_4 \cdot 2H_2O$, and Solution D contained 5.406 g/L $FeCl_3 \cdot 6H_2O$, and 7.4445 g/L Na_2EDTA. and maintained in a growth chamber set at 21°C and 16h/8h light/dark. Root temperature was maintained at 25°C by suspending the trays in a water bath.

The seedlings were uprooted from the sand when they reached growth stage 10 – 12 (Lancashire et al, 1991), rinsed and inoculated by dipping the roots into a suspension of *F. oxysporum* microconidia for one hour. The fungal isolates (521 and 522, Alberta Research Council collections) were originally isolated from symptomatic *B. napus* plants. Microconidia were generated by culturing each *F. oxysporum* isolate separately in 250 ml of modified Bilay's medium (Reid et al, 2003) for 7 – 14 days at 100 RPM, filtering and adjusting the suspensions to 1×10^5 microconidia per ml, then mixing the suspensions 1:1. After inoculation, the seedlings were returned to the sand cultures and incubated for 14 days. Canterra 1604 and SP Banner seedlings were included as susceptible and resistant controls, respectively.

Interaction phenotypes were determined using the scale given in Table 1 and Figure 2. Mean Disease Severity (MDS) was calculated for each genotype using the formula:

$$MDS = \frac{\sum ij}{\sum i}$$

where i was the number of plants in the jth severity category. FW resistance of each genotype was scored as resistant if MDS was less than three, susceptible if MDS was greater than or equal to seven; entries with MDS values outside these ranges were scored as intermediate.

Segregation and linkage analyses

Tissue samples for use in microsatellite procedures were collected from young leaves of parents and F_3 plants prior to inoculation. DNA was extracted using the CTAB method. PCR amplifications were performed using standard protocols (Naom et al, 1995) on an ABI 877 thermocycler/liquid handling robot (ABI Biosystems, Foster City, CA) or an MWG Primus HT multiblock thermocycler (MWG Biotech, Munich, Germany), using primer pairs specific to defined loci in the *Brassica* A and C genome. The primer pairs were fluorescently labelled with HEX (yellow), FAM (blue) and TET (green) and resulting PCR products were resolved on a MegaBACE 1000 96 capillary automated sequencer. Standard segregation analyses were performed using χ^2 tests to determine the mode of inheritance and number of genes involved in resistance. F_3 phenotypic data were used to infer whether the F_2 plant from which each F_3 line was derived was homozygous resistant or susceptible (i.e. no segregation among F_3 plants) or heterozygous (i.e. F_3 plants segregating for both resistance and susceptibility). The size of the PCR products was determined and primer pairs screened for polymorphisms using MegaBACE Fragment Profiler software (Amersham Biosciences). The linkage of marker loci and loci controlling resistance were determined with Mapmaker software (Lander et al, 1987). Recombination frequencies were converted to map distance (Kosambi, 1944).

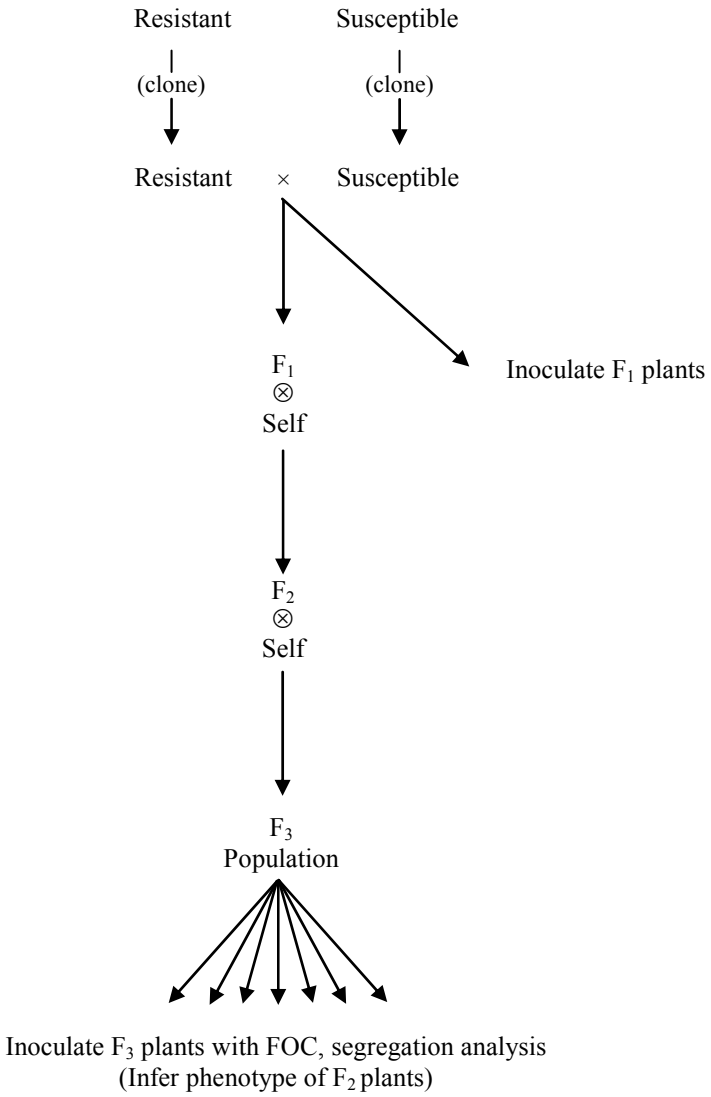

Figure 1 Generation and testing of *Brassica napus* populations segregating for Fusarium wilt resistance.

Table 1 Evaluation scale used to determine Fusarium wilt interation phenotype

		Symptoms	
Value	Stunting	Leaves and stems	Roots
1	None	None	normal
2	Slight	Slight Chlorosis, or intra-veinal yellowing	normal
3	Moderate	Chlorosis and intra-veinal yellowing expanding to third leaf	normal
5	Moderate	Same symptoms as of scale 3 + some necrosis+ some green tissue left	normal
7	Severe	Most tissue necrotic, remaining tissue chlorotic	brown
9	Severe	plant dead	brown

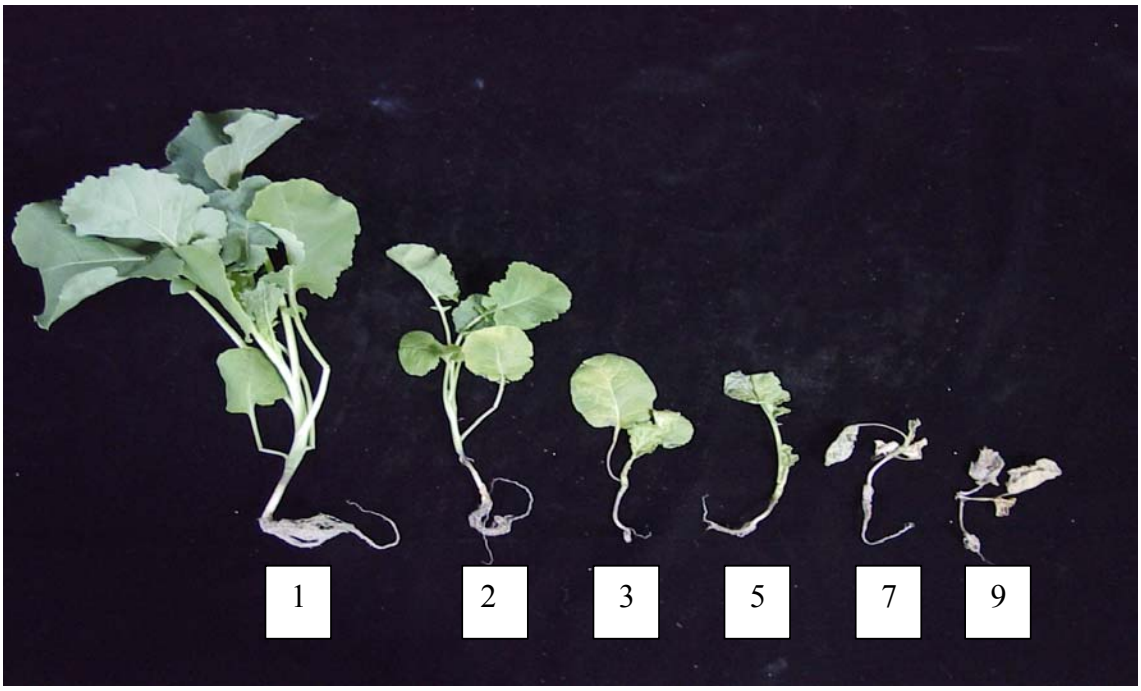

Figure 2 *Brassica napus - Fusarium oxysporum* interaction phenotypes, evaluated using the scale detailed in Table 1

Results and discussion

Segregation of resistance and susceptibility to *Fusarium oxysporum*

Phenotype tests of the parental genotypes confirmed that SP Banner and DH12075 were resistant, and Canterra 1604 was susceptible to fusarium wilt (Table 2), confirming that subsequently-derived mapping populations were the progeny of crosses between resistant and susceptible plants. Segregation of F_1 generation progeny (Table 2) indicated that all heterozygous F_1 progeny were phenotypically identical to the resistant parent, thus conforming to a dominance model for Fusarium wilt resistance. Segregation ratios of F1 plants from reciprocal crosses suggested that *F. oxysporum*–resistance is not affected by cytoplasmic factors (Table 2). No F_2 or backcross phenotypic data were obtained. Table 3 summarizes the segregation data for the F_3 lines obtained from self-pollinated individual F_2 plants based on MDS calculations for each line.

Segregation between F_3 lines (Table 3) were tested for fit to a monogenic dominant model. Chi-squared tests were used to test goodness-of-fit of the data in Table 3 to the expected 3:1 (R:S) F_2 segregation ratio for the combined resistant and intermediate scoring lines (R) against the susceptible lines (S). The Canterra 1604 × DH12075 and reciprocal crosses fit the expected 3R:1S ratio. The combined data for the two DH12075 populations (131R:39S) also fit a 3:1 ratio ($\chi = 0.38$; $p= 0.54$). These data strongly suggest that resistance in this cross was controlled by a single dominant gene for resistance to Fusarium wilt.

The observed segregation of the Canterra 1604 × SP Banner populations did not differ significantly ($\chi = 0.44$; $p= 0.51$) from a 13:3, but not a 3:1 segregation ratios (Table 3). However the reciprocal cross showed no segregation for susceptibility. These data are difficult to explain genetically, and accidental self-fertilization of the SP Banner parent cannot be ruled out. Barring accidental selfing, one theoretical possibility is suggested by the 13:3 segregation ratio, which implies control of the trait by a dominant gene in epistasis with a recessive suppressor. The reciprocal effect suggests that the suppressor may be a cytoplasmic. Dominant-recessive epistatic control of disease resistance with a maternal cytoplasmic factors has not been extensively documented in the literature, but has been found in stripe rust of wheat (Chen &Line, 1993) and barley (Chen &Line, 1999).

Resistance to fusarium wilt in *B. napus* appears to be controlled by a single dominant nuclear gene, assuming resistance in *B. napus* cv. DH12075 is representative of the trait in *B. napus* in general. In this sense, resistance in *B. napus* would be analogous to "Type A" monogenic resistance in *B. oleracea*. "Type A" resistance is race-specific in *B. oleracea*, implying that resistance to *F. oxysporum* may break down due to pathogen race change if the two resistances are indeed analogous. However, major gene resistance to *F. oxysporum* in *Brassica*, including *B. napus*, may prove stable over time. According to the assessment scheme of MacDonald &Linde, (2002), risk of *F. oxysporum*-resistance loss in *Brassica* is low because the pathogen's asexual mode of reproduction and the lack of a long-distance dispersal mechanism. This assessment is borne out by experience with *F.*

oxysporum on *B. oleracea*, for which only one instance of race change is known, despite the extensive cultivation of cole crops worldwide (Bosland &Williams, 1984; Bosland &Williams, 1986; Bosland &Williams, 1987a; Bosland &Williams, 1987b; Ramirez-Villupadua et al, 1985).

Linkage to microsatellite markers

Mapping of the resistance to Fusarium wilt was focussed on the Canterra 1604 × DH12075 cross because of the consistent 3R:1S ratios obtained with this cross. The first step in determining the map location of any genes associated with disease resistance was a microsatellite marker screen for polymorphism between the parental lines. Primers that spanned each chromosome of the A and C genomes in 5 cM intervals were organized into sets of three. Table 4 presents a summary of microsatellite primers from the A and C genomes tested on the parental DNA.

After screening 1263 primers, polymorphic primers were selected to begin mapping the F_3 population. Initially, a bulk segregant approach was used. Four R and four S F_3 were chosen from each cross. A 10ng/uL DNA stock was made for each line and pooled; the DNA set was then comprised of one R and one S sample for each cross. No polymorphisms were detected with this approach.

DNA from a subset of 15 resistant and 15 susceptible F_3 populations from the Canterra 1604 × SP Banner cross were then examined for polymorphic markers in the C-genome linkage groups N10,11,13,14,15,17,18 and 19. No microsatellite polymorphisms in the C genome could be associated with resistance, so the A genome was assessed. Linkage group N3 yielded a region where a polymorphism between SP Banner and Canterra 1604 was linked with the resistant phenotype. The putative locus was named FusR1. The primer pair sR660 (located at 140cM on the *B. napus* consensus map) gave the only strong association for this population. A more intensive screen of N3 was then conducted using DNA from the Canterra 1604 × SP Banner subset of F_3 lines, and also another subset comprised of DNA from F_3 lines from the Canterra 1604 × DH12075 cross. The resulting linkage map is presented as Figure 3.

The N3 linkage group is part of the *B. napus* A-genome, originating from *B. rapa*. The discovery of Fusarium wilt resistance on a *B. rapa* chromosome was unexpected, since resistance to *F. oxysporum* is best characterized in *B. oleracea* . However, resistance to *F. oxysporum* has also been identified in *B. rapa* (Fjellstrom &Williams, 1997). The presence of Fusarium wilt resistance in the A-genome of *B. napus* and the C-genome (in *B. oleracea* and perhaps also in *B. napus*) suggests that at least two sources of resistance are available to *B. napus* breeders, via interspecific crosses or within *B. napus*. Combining resistances could be used to increase the longevity of resistance to Fusarium wilt.

The association between FusR1 and the N3 polymorphic markers (including those in the interval shown in Figure 3) could be refined by analyzing the entire Canterra 1604 × SP Banner F_3 population. We expected that marker sR536 would not remain coincident with FusR1, and that the map interval between sR536 and sR634OI that spans the FusR1

locus will be significantly expanded when a complete map based on this population is obtained.

Table 2 Interaction phenotype data from parental and F_1 lines of *Brassica napus* crosses for Fusarium wilt resistance.

Genotype	Generation	Observed [a]			Expected	
		R	I	S	R+I	S
Canterra 1604	P	0	4	16	0	20
DH12075	P	20	0	0	20	0
SP Banner	P	20	0	0	20	0
Canterra 1604 × DH12075	F_1	20	0	0	20	0
DH12075 × Canterra 1604	F_1	20	0	0	20	0
SP Banner × Canterra 1604	F_1	109	5	2	116	0
Canterra 1604 × SP Banner	F_1	69	9	0	78	0

[a] Observed number of plants in each phenotype class. Plants with mean disease severity (MDS) ≤ 2.5, $3-7$, or ≥ 7.5 were classified as resistant (R), intermediate (I) or susceptible (S), respectively.

[b] Expected segregation of combined resistant and intermediate scoring lines (R and I) against susceptible lines (S) for resistance or susceptibility under the hypothesis that fusarium wilt resistance is monogenic and dominant over susceptibility.

Table 3 Interaction phenotype data from F3 lines of *Brassica napus* crosses for Fusarium wilt resistance

Cross	F_3 segregation[a]			F_2 [b]	χ^{2c}	P [c]
	R	I	S			
Canterra 1604 × SP Banner	46	37	16	S	1.7294	0.0423
SP Banner × Canterra 1604	92	1	0	H	31.0000	<0.0001
Cant 1604 × DH12075	31	31	23	S	0.1922	0.6611
DH12075 × Canterra 1604	40	29	16	S	1.7294	0.1885

[a] Interaction Phenotype, i.e. the number of plants in each phenotype class. Plants with mean disease severity (MDS) ≤ 2.5, $3-7$, or ≥ 7.5 were classified as resistant (R), intermediate (I) or susceptible (S), respectively.

[b] S= segregating, H= homozygous

[c] χ^2-value and probability that the observed segregation ratio for resistance or susceptibility is not different from from a 3:1 ratio of combined resistant and intermediate scoring lines (R and I) against susceptible lines (S).

Table 4. Polymorphism among SSR markers from each linkage group (N1 – N19) among *Brassica napus* parental genotypes Canterra 1604, SP Banner, and DH12075

Linkage group	Genome	Monomorphic	Polymorphic	Failed or incomplete amplification	Total	% Polymorphic
N1	A	20	15	29	64	23
N2	A	19	12	16	47	26
N3	A	22	27	37	86	31
N4	A	15	16	14	45	36
N5	A	8	3	22	33	9
N6	A	12	12	33	57	21
N7	A	16	12	20	48	25
N8	A	19	2	13	34	6
N9	A	37	14	35	86	16
N10	C	21	10	25	56	18
N11	C	27	17	31	75	23
N12	C	32	21	51	104	20
N13	C	55	28	37	120	23
N14	C	37	41	22	100	41
N15	C	35	9	6	50	18
N16	C	10	6	8	24	25
N17	C	31	11	33	75	15
N18	C	37	13	25	75	17
N19	C	25	20	39	84	24
Total		478	289	496	1263	

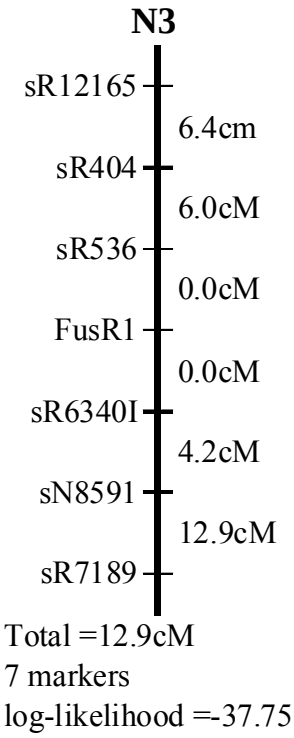

Figure 3 Linkage map of Canterra 1604 × DH12075 microsatellite markers on *Brassica napus* linkage group N3 associated with Fusarium wilt resistance.

Conclusions

Resistance to Fusarium wilt of *Brassica napus* is conferred by a single, dominant gene associated with linkage group N3, based on segregation and linkage analysis of a Canterra 1604 × DH12075 cross. Closely-associated SSR markers were identified.

Implications of the study

This study will allow member-organizations of the Microsatellite Marker Consortium to screen accessions for resistance/susceptibility to fusarium wilt. The consortium includes most of the oilseed *Brassica napus* breeding organizations active in the North American marketplace. Members have access to the PCR primer sequences associated with the marker loci identified in Figure 3. Currently, breeding organizations either screen selections in naturally-infested field nurseries, or test for resistance in controlled-environment tests. Use of the markers would allow these organizations to screen large numbers of accessions, and also avoid much of the field- or controlled environment screening phenotype testing that is currently necessary. The procedures of the Western Canada Canola/Rapeseed Recommending Committee require that breeders submit proof that candidate cultivars have been tested for Fusarium wilt susceptibility, so some phenotype testing is likely to continue, but only for lines being considered for variety registration.

Dr. S. R. Rimmer at AAFC-Saskatoon was unable to refine the linkage map in Figure 3 by screening the entire F_3 population before his death in September 2008. Completion of this work would produce more robust linkage to SSR markers, and also define the most-closely linked markers with publically-available primer sequences.

Identification of resistance on the *B. napus* A-genome (*B. rapa*) suggests that two major-gene resistances (the other being the "Type-A" resistance from *B. oleracea*) are available to *B. napus* breeders, in addition to quantitative "Type-B" resistance, also from *B. oleracea*. These genes can be combined to make Fusarium wilt resistance more stable in *B. napus*, an important consideration since loss of resistance would result in severe yield losses (Lange et al, 2007) in affected fields.

Publications arising from the project

1. Doctoral dissertation (in preparation), Humboldt University of Berlin, Germany
2. Peter, M. 2008. Untersuchungen zur Pathogenität von *Fusarium oxysporum*-Isolaten an Winterrapssorten [Studies on the pathogenicty of *Fusarium oxysporum* isolates from winter rapeseed] B.Sc. dissertation, Humboldt University of Berlin, Germany. (Methodology developed for this project applied to studies conducted B.Sc. student supervised by R. Lange)

Biographical data

Name: **Last Name:** Lange **First Name:** Ralph

Position / Organization / Dept.: Research Scientist

Address: P. O. Bag 4000, Highway 16A and 75 Street | **City :**Vegreville **Prov.:**AB **Postal Code:**T9C 1T4

E-mail: Ralph.Lange@arc.ab.ca

Phone: (780) 632-8226 | **Fax:** (780) 632-8612

Past experience relevant to project: (Point form, concise.)

- Program Leader, Phytochemicals Program, Bioresource Technologies Business Unit, Alberta Research Council. Primary project: Production, extraction and commercialization of artemisinin from Alberta-grown *Artemisia annua*.
- Project leader, Sclerotinia Stem Rot Field Nursery Evaluation of Resistance and Fungicide Efficacy, Agriculture and Food Council #2006F068R, Canola Agronomic Research Program #CARP2006-07
- Project leader, Monitoring and controlling fusarium wilt of canola, Alberta Crop Industry Development Fund /Agriculture and Food Council – CARD Program #2003A314R
- Project leader, Fusarium wilt of canola: development of effective control measures, Canola Agronomic Research Program AG#2002-01-10 (2001-2003)
- Project leader, Identification and Quantification of a New Canola Wilt in Western Canada, Farming For The Future Matching Grants Research Program Project #2000M633

Degrees / Certificates / Diplomas:	**Institution:**	
B. Sc (Agriculture)	University of Alberta	(1988)
M.Sc. (Plant pathology)	University of Alberta	(1993)
Doktorand (Ph.D. Candidate), Phytomedicine	Humoldt University of Berlin	(ongoing)

Publications and Patents:

Fitt, B.D.L., Hu, B., Li, Z., Liu, S.-Y., **Lange, R.M.**, Kharbanda, P.D. & White, R.P. 2008. Strategies to prevent spread of *Leptosphaeria maculans* (phoma stem canker) onto oilseed rape crops in China. *Plant Pathology*, 57(4):652-664.

Lange, R.M., Goßmann, M. & Büttner, C. 2007. Yield loss in susceptible cultivars of spring rapeseed due to fusarium wilt caused by *Fusarium oxysporum* . *Communications in Agricultural and Applied Biological Sciences, Ghent University*, 72:723-734.

Lange, R. M., Goßmann, M., and Büttner, C. 2006. Untersuchungen zur Identität, Pathogenität und genetischen Diversität von *Fusarium oxysporum* an Sommerraps in Kanada. 55. Deutsche Pflanzenschutztagung, Göttingen, Germany, 25-28 Sept. 2006. Heft 400, pp 180-181.

Tewari, J. P., S. E. Strelkov, D. Orchard, M. Hartman, **R. M. Lange**, and Turkington T. K. 2005. Identification of clubroot of crucifers on canola (*Brassica napus*) in Alberta. Can. J. Plant Pathol. 27:143-144.

Cathcart, R. J., K. Topinka, P. D. Kharbanda, **R. M. Lange**, and L. M. Hall. 2005. Effect of rotation length and canola (*Brassica napus*) variety on weed populations and canola yield. Weed Science.

Lange, R. M., D. Benard, B. D. Gossen, A. C. Diener, and W. Dmytriw. 2004. Etiology, epidemiology and genetic resistance to fusarium wilt of canola. Can. J. Plant Pathol. 26:227.

Kenward, K. D., K. S. Zaychuk, T. A. McLafferty, R. Stevens, and **R. M. Lange**. 2004. Comparative evaluation of *Fusarium graminearum* detection methods. Can. J. Plant Pathol. 26:226.

# of Refereed papers: 3	**Conference proceedings:** 9
Relevant Patents obtained: 0	**Other relevant citations:** 22

Reference List

1. Benard, D., Bernier, J. & Lange, R.M. (2003) Survey of fusarium wilt and other canola diseases in Alberta in 2002. *Canadian Plant Disease Survey*, **83**, 94-95.

2. Benard, D., Lange, R.M. & Byer, R. (2002) Survey of fusarium wilt and other diseases of canola in Alberta, 2001. *Canadian Plant Disease Survey*, **82**, 80-81.

3. Benard, D., Lange, R.M. & Harrison, L.M. (2001) Survey of fusarium wilt and other canola diseases in Alberta, 2000. *Canadian Plant Disease Survey*, **81**, 102-104.

4. Blank, L.M. (1937) Fusarium resistance in Wisconsin All Seasons cabbage. *J. Agric. Res.* **55**, 497-510.

5. Bosland, P.W. & Williams, P.H. (1984) A new pathotype of the crucifer yellows organism capable of causing disease in cool soils. *Cruciferae Newsletter*, 95.

6. Bosland, P.W. & Williams, P.H. (1986) A survey of resistance to *Fusarium oxysporum* f. sp. *conglutinans*, race 2. In Williams, P.H. (ed), *Crucifer genetics cooperative resource book. I.D. DF0C2R 5-22-87*. University of Wisconsin, Madison, Wisconsin.

7. Bosland, P. W. and Williams, P. H. An evaluation of *Fusarium oxysporum* from crucifers based on pathogenicity, isozyme polymorphism, vegetative compatibilty, and geographic origin. Canadian Journal of Botany 65, 2067-2073. 1987.

8. Bosland, P.W. & Williams, P.H. (1987b) Sources of resistance to *Fusarium oxysporum* f. sp. *conglutinans*, race 2. *HortScience*, **22** , 669-670.

9. Chen, X.M. & Line, R.F. (1993) Inheritance of stripe rust resistance in wheat cultivars postulated to have resistance genes at Yr3 and Yr4 loci. *Phytopathology*, **83**, 382-388.

10. Chen, X.M. & Line, R.F. (1999) Recessive Genes for Resistance to Puccinia Striiformis F. Sp. Hordei in Barley. *Phytopathology*, **89**, 226-232.

11. Dmytriw, W. & Lange, R.M. (2005) Survey of canola diseases in Alberta, 2004. *Canadian Plant Disease Survey*, **85**, 65-71.

12. Fall, A.L., Byrne, P.F., Jung, G., Coyne, D.P., Brick, M.A. & Schwartz, H.F. (2001) Detection and Mapping of a Major Locus for Fusarium Wilt Resistance in Common Bean. *Crop Science*, **41**, 1494-1498.

13. Fjellstrom, R.G. & Williams, P.H. (1997) Fusarium yellows and turnip mosaic virus resistance in *Brassica rapa* and *B. juncea*. *HortScience*, **32**, 927-93.

14. Gaetan, S.A. (2005a) First Outbreak of Blackleg Caused by *Phoma lingam* in Commercial Canola Fields in Argentina. *Plant Disease*, **89**, 435.

15. Gaetan, S.A. (2005b) Occurrence of Fusarium Wilt on Canola Caused by *Fusarium oxysporum* f. sp *conglutinans* in Argentina. *Plant Disease*, **89** , 432.

16. Gaetan, S. A. Fusarium wilt, Canola - Argentina: 1st Report. ProMED-mail post . April 14, 2005. International Society for Infectious Diseases.

17. Halila, I., Cobos, M.J., Rubio, J., Millan, T., Kharrat, M., Marrakchi, M. & Gil, J. (2009) Tagging and Mapping a Second Resistance Gene for Fusarium Wilt Race 0 in Chickpea. *European Journal of Plant Pathology*, **124**, 87-92.

18. Hamwieh, A., Udupa, S.M., Choumane, W., Sarker, A., Dreyer, F., Jung, C. & Baum, M. (2005) A Genetic Linkage Map of Lens Sp Based on Microsatellite and Aflp Markers and the Localization of Fusarium Vascular Wilt Resistance. *Theoretical and Applied Genetics*, **110**, 669-677.

19. Kosambi, D.D. (1944) *Annals of Eugenics*, **12**, 172-175.

20. Lancashire, P.D., Bleiholder, H., Van den Boom, T., Langelüddeke, P., Stauss, R., Weber, E. & Witzenberger, A. (1991) A uniform decimal code for growth stages of crops and weeds. *Annals of applied biology*, **119**, 561-601.

21. Lander, E.S., Green, P., Abrahamson, J., Barlow, A., Daly, M.J. & Lincoln, S.E.N.L. (1987) Mapmaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural population. *Genomics*, **1**, 174-181.

22. Lange, R.M. & Franke, C. (2004) Fusarium wilt of canola in Alberta and Saskatchewan in 2003. *Canadian Plant Disease Survey*, **84**, 92-95.

23. Lange, R.M., Goßmann, M. & Büttner, C. (2007) Yield loss in susceptible cultivars of spring rapeseed due to fusarium wilt caused by *Fusarium oxysporum* . *Communications in Agricultural and Applied Biological Sciences, Ghent University*, **72**, 723-734.

24. Lange, R. M., Harrison, L. M., and Kharbanda, P. D. A new Fusarium wilt of canola in Alberta, Canada in 1999. Joint Meeting of the Canadian Phytopathological Society and the Pacific Division of the American Phytopathological Society, June 18-21,2000, Victoria, BC, Canada. p. 25 (vii-34). 2000. Victoria, BC, Canadian Phytopathological Society.

25. Lim, G.T.T., Wang, G.P., Hemming, M.N., Basuki, S., Mcgrath, D.J., Carroll, B.J. & Jones, D.A. (2006) Mapping the I-3 Gene for Resistance to Fusarium Wilt in Tomato: Application of an I-3 Marker in Tomato Improvement and Progress Towards the Cloning of I-3. *Australasian Plant Pathology*, **35**, 671-680.

26. MacDonald, B.A. & Linde, C. (2002) Pathogen population genetics, evolutionary potential, and durable resistance. *Annual Review of Phytopathology*, **40**, 349-379.

27. McLaren D. L., Graham, T.L., Kaminski, D.A. & Lange, R.M. (2005) Canola diseaes in Manitoba: Distribution, prevalence and incidence in 2004. *Canadian Plant Disease Survey*, **85**, 76-77.

28. McLaren D. L. & Platford, R.G. (2001) Distribution, prevalence and incidence of canola diseases in Manitoba (2000). *Canadian Plant Disease Survey*, **81**, 106-110.

29. McLaren D. L., Platford, R.G., Lamb J. L. & Kaminski, D.A. (2003) Canola Diseases in Manitoba: Distribution, prevalence and incidence in 2002. *Canadian Plant Disease Survey* , **83**, 96-98.

30. Mutlu, N., Boyaci, F.H., Gocmen, M. & Abak, K. (2008) Development of SRAP, SRAP-RGA, RAPD and SCAR Markers Linked With a Fusarium Wilt Resistance Gene in Eggplant. *Theoretical and Applied Genetics*, **117**, 1303-1312.

31. Naom, I.S., Mathew, C.G. & Town, M.M. (1995) Genetic mapping with microsatellites. In Glover, D.M. & Hames, B.D. (eds), *NA Cloning: A Practical Approach Volume 3: Complex Genomes*. I.R.L. Press, Oxford, pp. pp. 195-217.

32. Nikonorenkov V.A., Portenko L.G. & Korpatchev, V.V. (1996) Fusarios Rapsa. *Mikologiya i Fitopatologiya*, 45.

33. Oumouloud, A., Arnedo-Andres, M.S., Gonzalez-Torres, R. & Alvarez, J.M. (2008) Development of Molecular Markers Linked to the Fom-1 Locus for Resistance to Fusarium Race 2 in Melon. *Euphytica*, **164**, 347-356.

34. Pearse, P.G., Morrall, R.A.A., Kutcher, H.R., Kaminski, D.A., Duczek L.J., Gugel, R.K., Rimmer, S.R. & Berard, R. (2000) Survey of canola diseases in Saskatchewan, 1999. *Canadian Plant Disease Survey*, **80**, 81-82.

35. Pearse, P.G., Morrall, R.A.A., Kutcher, H.R., Keri, M., Kaminski, D., Gugel, R.K., Anderson, K., Trail, C. & Greuel, W. (2001) Survey of canola diseases in Saskatchewan, 2000. **81**, 105-107.

36. Pearse, P.G. , Morrall, R.A.A., Kutcher, H.R., Yasinowski, J.M., Gugel, R.K., Bassendowski , K.A. & Cowell, L.E. (2004) Survey of canola diseases in Saskatchewan, 2003. *Canadian Plant Disease Survey*, **84**, 96-97.

37. Pearse, P.G., Morrall, R.A.A., Kutcher, H.R., Yasinowski, J.M., Harris C.L., Gugel, R.K. & Bassendowski , K.A. (2005) Survey of canola diseases in Saskatchewan, 2004. *Canadian Plant Disease Survey*, **85**, 74-75.

38. Pearse, P.G., Weir, T.N., Meachem, E. & Morrall, R.A.A. (2003) Fusarium wilt and other diseases of canola in Saskatchewan, 2002. *Canadian Plant Disease Survey*, **83**, 99-100.

39. Portenko, L.G. (1998) Virulence and Vegetative Compatibility of *Fusarium oxysporum* Schlecht.: Fr. Isolates From Brassica Napus and Allied Species of Brassicaceae Family. *Mikologiya I Fitopatologiya*, **32**, 71-75.

40. Ramirez-Villupadua, J., Endo, R.M., Bosland, P.W. & Williams, P.H. (1985) A new race of *Fusarium oxysporum* f. sp. *conglutinans* that attacks cabbage with type A resistance. *Plant Disease*, **69**, 612-613.

41. Reid, L. M., Hamilton, R. I., and Mather, D. E. (2003) Technical Bulletin 1996-5E: Screening Maize for Resistance to Gibberella Ear Rot. http://res2.agr.ca/ecorc/gibberella/index_e.htm. Retrieved Februrary 26, 2007.

42. Walker, J.C. (1926) Studies upon the inheritance of Fusarium resistance in cabbage. *Phytopathology* , **16**, 87 (Abstract).

43. Walker, J.C. (1930) Inheritance of *Fusarium* resistance in cabbage. *J. Agric. Res.* **40**, 721-745.