Identification and Quantification of a New Canola Wilt in Western Canada

Canola Agronomic Research Program AG#2000-6

Final Project Report

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January 31, 2002

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ACKNOWLEDGEMENTS

The authors gratefully acknowledge financial support from the Canola Council of Canada and the Alberta Agricultural Research Institute (AARI). The assistance of Julie Bernier, Deanna Deaville, Wendi Eriksen, Mike Hall, Lorraine Harrison, Kent MacDonald, Jeff Newman, Michelle Sulz, and the Cereal and Oilseed Specialists and Scientists who participated in the survey is greatly appreciated. We also thank Randy Clear and the Canadian Grain Commission, Winnipeg, MB for identification of selected isolates.

ABSTRACT

A wilt disease previously unreported in North America was observed in the Peace River and north east regions of Alberta beginning in 1999. The disease induced chlorosis, stem necrosis, vascular discolouration and premature desiccation in *Brassica napus* and *B. rapa*. This disease could become a serious threat to canola production in Canada.

The objectives of this research were to identify the causal agent of wilt on canola; to determine its host range; to determine its race (if applicable, for breeding purposes); and to determine if any current canola cultivars are particularly resistant or susceptible.

Isolations from diseased tissue indicated that *Fusarium avenaceum* was the causal agent, and these isolates showed a high degree of virulence. Host range experiments showed that *F. avenaceum* has a wide host range, and is capable of surviving on a wide range of crop, forage and weed species. The pathogen produced pectolytic enzymes, which may be implicated in the mode of action. Differences in cultivar susceptibility were demonstrated, with Nexera 705 being highly susceptible. Several attempts to develop a simple and reliable cultivar screening method were made, but the lack of corresponding field data limited this approach. In 2000, a province-wide disease survey was conducted, which documented the occurrence of Fusarium wilt and other canola diseases. A replicated field experiment also showed that Fusarium wilt can cause significant yield losses in canola.

INTRODUCTION

In the fall of 1999, canola producers and Alberta Agriculture, Food and Rural Development Cereal and Oilseed Specialists began noticing that canola plants in some fields in the areas surrounding Fort Vermilion and Vegreville were exhibiting unusual symptoms. Affected plants were discoloured and pods had not filled properly. These symptoms differed from other canola diseases: roots of affected plants were intact, unlike in root rot; no Blackleg-like cankers were noticed; and the stem shredding, sclerotia and lodging typical of Sclerotinia stem rot were absent. The disease induced chlorosis, stem necrosis, vascular discolouration and premature desiccation in *Brassica napus* and *B. rapa*. Fields were found where up to 29% of all canola plants were diseased. Yield was severely reduced by the disease; fully- and partially-wilted plants yielded 0.2% and 19.3% of asymptomatic plants, respectively.

The symptoms of the disease strongly resembled another wilt disease caused by fungi of the genus *Verticillium* (Heale and Karapapa 1999). *Verticillum dahliae* is a serious pathogen of oilseed rape in Europe (Heale and Karapapa 1999; Zeise 1992), and *V. albo-atrum* has caused disease on rutabaga in California and on *B. rapa* in Kansas (Farr et al. 1989), but has not been recorded on canola plants (*Brassica napus* var. *napus, and B. rapa* subsp. *oleifera*) in North America. However, an unidentified *Verticillium* species has been isolated from rutabaga (*B. napus* var. *napobrassica*) in Quebec (Ginns 1986). Verticillium wilt also occurs on North American vegetable crops such as cauliflower (Ginns 1986).

Repeated attempts to recover *Verticillium* spp. from symptomatic plants using semi-selective media failed to result in the isolation of *Verticillium* spp, but repeatedly resulted in the isolation of *Fusarium* spp. For this reason, it was suspected that the causal agent might be *Fusarium oxysporum*, since this fungus causes most Fusarium wilt dieases worldwide. For example, *Fusarium oxysporum* has caused "yellows" or wilt of *Brassica napus* in North Carolina, and *Fusarium oxysporum* f. sp. *conglutinans* (FOC) has attacked *B. rapa* in Indiana, Misisissippi and Texas (Farr et al. 1989). Of particular interest to Canadian plant pathologists and plant breeders is a FOC race capable of infecting *Brassica* plants under the cool soil temperatures that occur in Canada (Bosland and Williams 1984; Bosland et al. 1988). *Fusarium oxysporum* has been isolated from rapeseed roots in Alberta (Berkenkamp and Vaartnou 1972), but has not been associated with wilt symptoms. FOC has not been isolated from oilseed Brassicas in Canada, however the fungus has caused wilt on cabbage and cauliflower in Ontario (Ginns 1986). *Fusarium oxysporum* has caused serious wilt of *B. juncea* in India; the fungus was also found to be highly pathogenic to *B. rapa* and *Raphanus sativus* (radish) (Gupta 1973; Rai and Singh 1973).

It is very possible that this disease could become a serious threat to canola production in Canada. Many important diseases can be traced back to a few scattered reports, only to later become major problems. For instance, Blackleg of canola was identified in a few fields in Saskatchewan in 1975, and since then has become an extremely serious disease across Canada (Gugel and Petrie 1992). Fusarium head blight

of cereals, now a serious threat to western Canadian wheat and barley producers, was rare in Manitoba prior to 1985, when two infected fields were identified (Clear and Abramson 1986; Gordon 1952). This new wilt is a potential threat that requires an immediate response so that timely action can be taken.

Fusarium wilt of canola has not been previously described; therefore, this project was udertaken to begin some of this work. Even though Fusarial wilts are usually caused by *F. oxysporum*, the true causal agent needed to be identified and confirmed. The ability of other crop and weed species to act as alternative hosts needed to be determined as a prerequisite to the development of cultural control methods. An important source of control may exist in some level of natural resistance within the crop itself, so the possibility of susceptibility differences among canola cultivars required investigation. This project was undertaken to develop a clearer understanding of this new disease of canola, in an effort to be proactive.

OBJECTIVES

1. To identify the causal agent of wilt on canola.
2. To determine its host range.
3. To determine its race (if applicable, for breeding purposes).
4. To determine if any current canola cultivars are particularly resistant or susceptible.

MATERIALS AND METHODS

Isolations

Isolation from stems

Studies were undertaken to determine the causal agent of canola wilt. Isolations were made from both fresh and dried diseased tissue from naturally infected canola grown in 1999 and 2000, from six different sampling locations in Alberta. Samples were taken from three locations in 1999 (Fort Vermilion, Vegreville, and Peace River), and three locations in 2000 (two separate sites near Ranfurly, and one near Strome).

In 1999, 0.5 cm^3 stem piece were removed from the middle section (ca. 20-30 cm above the soil line) of symptomatic plants, surface sterilized by soaking for 60 seconds in 10% bleach solution, and plated onto 2.0% Difco brand water agar, 3.9% Difco brand potato-dextrose agar (PDA), V-8 agar amended with 500 ppm each of Streptomycin sulphate and Penicillin-G (Sigma), and Peptone PCNB (PPA/Nash-Snyder) (N-S) *Fusarium*-semi-selective medium (Burgess et al. 1994). Representative fungal cultures were purified by single spore isolation. Pure cultures were transferred to PDA and carnation leaf agar (CLA) and identified using published methods (Kharbanda and Stevens 1988; Burgess et al. 1994).

In 2000, both the lower stem (just above the soil line) and the middle section of the stem (ca. 20-30 cm above the soil line) were sampled, and two isolation methods were used. Method 1 consisted of surface sterilizing 0.5 cm^3 pieces of infected canola stems by immersing them for two seconds into 10% bleach, air-drying briefly, then cutting up into small pieces (ca. 0.5 cm^3). Half of these isolations were rinsed with sterile distilled water before air-drying, and half were not. All pieces were then placed onto N-S medium in 15 × 150 mm Petri dishes and incubated at room temperature (ca. 21 °C) on a laboratory bench until fungal mycelium began to emerge. Method 2 was identical to Method 1, except stem pieces were surface-sterilized by blotting with cotton soaked in 95% ethanol. All plated samples were incubated at a constant temperature of 25 °C and 14 h daylength with fluorescent illumination. Twenty-four representative colonies (12 for each method) were transferred to PDA and CLA for identification.

Isolation from seed

Isolations were also made from seed harvested from severely affected canola plants grown in the field in 1999 and in 2000 (from Ranfurly). Seed from plants harvested from uninfected fields was also included as a control. In 1999, seeds were soaked for 60 seconds in 10% bleach, 10 seeds per sample were then plated onto 2.0% water agar amended with 500 ppm each of Streptomycin sulphate and Penicillin-G in 150 x 15 mm Petri dishes. Four to six replicate plates were prepared for each sample. This experiment

was repeated twice. In 2000, three isolation methods were used. Method 1 consisted of plating untreated seeds (five per plate) directly onto N-S media. Method 2 involved immersing the seeds for two seconds into 10% bleach, drying on sterile filter paper, and then plating the seeds onto N-S media. Method 3 was the same as Method 2, but seeds were immersed in bleach for 60 seconds.

Pathogenicity Experiments

Root dip inoculation method

In 1999, the pathogenicity of *Fusarium* spp. isolates obtained from symptomatic plants collected near Vegreville, Vermilion and Ft. Vermilion, Alberta, were tested in inoculation experiments. Stem tissue was collected from the middle section of the sampled plants, the outer epidermal layer and the stem pith were carefully excised, then surface-sterilized in 10% bleach and incubated on N-S medium on a laboratory bench until mycelial growth was detected. Mycelium was transferred to PDA and CLA for species identification and purification through single spore transfer. Inocula were prepared by growing five representative isolates on PDA for one week, and then transferring to potato dextrose broth (PDB) on a shaker at 200 RPM and 25 °C for one week. The broth cultures were lightly macerated for 10 seconds in a Waring blender, and then filtered through two layers of sterile cheesecloth to remove large mycelial fragments. Macro- and microcondia and mycelial fragments were quantified using a haemocytometer and adusted to the required concentration with sterile distilled water. The inoculated plants were returned to pots containing soil-less mix and placed in the growth chamber with 16 hrs light at 22 °C and 8 hrs dark at 20 °C. Plants were observed for disease development for up to one month post-inoculation.

To prepare plants for inoculation, seed from *Brassica napus* cv. Quest was surface sterilized in 10% bleach for 60 seconds, dried on sterile filter paper, then planted into ten inch plastic pots containing well-moistened Sunshine brand soilless mix. Pots were then placed in a growth chamber programmed to provide 14 h of daylight at 23 °C/18 °C (day/night) and 80% relative humidity until plants had reached the required growth stage for inoculation.

A clipped root dip method was used to inoculate the plants. The plants were carefully uprooted and the roots gently washed. The roots were then clipped with scissors to a length of 3-7 cm (roots were left longer for older plants to minimize transplantation shock), and immediately placed into the prepared inoculum suspension. Plants were left in the inocula for approximately 4 hours, then transplanted into 10-inch plots and placed into a growth chamber programmed for the conditions detailed above.

Two experiments were conducted: 1) a comparison of pathogenicity among the five isolates; and 2) A study of the interaction between inoculum concentration and inoculation stage.

Root dip pathogenicity test

Seven isolates were tested against canola plants in the three-leaf growth stage (BBCH stage 13 (Lancashire et al. 1991)). Choice of *Fusarium* isolates was based on colony morphology and pigmentation, without *a priori* knowledge of species identity. Species identification was completed after the root dip pathogenicity test was complete. Five plants were transplanted to each pot after inoculation, and four replicate pots were prepared per isolate. A completely randomized design was used. Plants treated with non-inoculated medium were included as a control. To evaluate virulence of the isolates, the number of asymptomatic plants per pot was counted 42 days after inoculation.

Inoculation stage

Plants in each of the one to seven leaf stages (BBCH stages 11-17 (Lancashire et al. 1991)) were inoculated using the methods described above. A total of eight inocula were prepared for the experiment: four subcultures of *F. avenaceum* isolate F5-3, two subcultures of *F. avenaceum* isolate F6, *F. oxysporum* isolate "Quest 875" and sterile PDB medium as an non-inoculated control. After inoculation, five plants were transplanted to each pot. Four replicate pots were prepared for each combination of inoculum and plant growth stage. Pots were arranged in a completely randomized design. The inoculated plants were incubated in a growth chamber under the conditions described above for 15 days. Fusarium wilt severity was assessed at the end of the experiment for each plant using a simple 0-2 scale, where 0=no disease, 1=intermediate symptoms and 2= complete wilted or dead plant. Supplemental measurements of disease severity including plant height and dry weight biomass, and the number of leaves on each plant were also collected.

Stem inoculation

Brassica napus cv. Quest plants at BBCH growth stage 34 were inoculated using three different stem inoculation methods. Three inoculation methods were used on each plant: i) injecting approximately 100μl of 1 x 10^6 macroconidiospore suspension into the first internode; ii) severing the petiole of the fourth true leaf, applying a cotton swab throughly wetted with approximately 5ml of the same macroconidiospore suspension and wrapping the swab, petiole and adjacent stem area with Parafilm M sealing film (American National Can, Menasha, WI); and iii) making a small (ca. 5mm) wound near the third node, inserting a small amount of *F. avenaceum* mycelium and wrapping the

wound with wet cotton and Parafilm. Macroconida from three *F. avenaceum* isolates were used to prepare inocula. The inoculated plants were incubated in a growth chamber set at 20° C/18° C day/night temperature with a 16h diurnal cycle. Five plants were grown per 6-inch pot in Sunshine® soilless medium. Plants were arranged in a completely randomized design after inoculation. Cotton and Parafilm was removed six days after inoculation. Disease severity was quantified by visually estimating the percentage stem girdling on each plant 17 days after inoculation.

Seed Germination Tests

In 1999 and 2000, germination tests on agar were performed on seed from highly infected, moderately infected, and uninfected plants of the canola cultivar Nexera 705. Seeds were surface sterilized by soaking in 10% bleach solution for 60 seconds, and then plated onto dual water agar (10 seeds per plate) and placed at approximately 22C for five days, after which they were rated for germination.

In 2000, germination in soil was also tested using seeds from highly infected, moderately infected, and uninfected Nexera 705 plants and seeds from infected and uninfected Quantum canola plants. Seeds were planted into root trainers and placed in the growth chamber with 16 h daylight at 20C and 8 h dark at 18C, all at 80% relative humidity. After nine days, the number of emerged plants was counted.

Host Range Experiments

In 2001, seeds from selected crop, forage and weed species were planted into soil-less Sunshine® mix in root trainers and grown in a growth chamber with 14 hrs light at 23C and 10 hrs dark at 18C all at 80% relative humidity. At a stage of 4 – 6 leaves or 10 to 15 cm in height, plants were carefully uprooted, the roots were cut with scissors so that the remaining root was 1.0 to 1.5 cm in length, and the plants were placed in spore suspensions of each of several *Fusarium* spp. (Figure 1). The *F. avenaceum* isolates were obtained at the ARC from Fusarium wilt-infected canola in 1999 and 2000. The *F. oxysporum* and *F. graminearum* isolates obtained from the Eastern Cereal and Oilseed Research Centre (ECORC) in Ottawa were chosen as positive controls. Check (negative control) plants were treated as described above, except they were placed in sterile PDB. The isolates were grown on PDA for one week, and then 5 plugs of each isolate were placed into flasks of PDB on a shaker at 200 rpm and 25C for two weeks. The spore suspensions were made by blending the PDB in a Waring blender for 10 seconds, and diluting the suspension as necessary with sterile distilled water to approximately 1.0 x 10^7 spores/hyphal pieces per ml (determined using a haemocytometer). After 3 to 4 hours of soaking in the spore suspensions, the inoculated plants and the control plants were planted into 10-inch pots (3 plants of the same treatment per pot) containing Sunshine brand soil-less mix and returned to the growth chamber. The inoculum that the roots had been soaking in (about 20 ml) was poured into the respective pots to

provide additional inoculum. Plants were observed for disease for 3 weeks post-inoculation.

After three weeks, plant roots and stems were surface sterilized by dipping into 10% bleach solution for two seconds, and then rinsing in sterile distilled water. The tissue was allowed to air dry briefly, and then cut into pieces approximately 0.5 cm in length. Pieces from the roots, lower stems, and upper stems were plated onto N-S media and placed in the growth chamber (14 h: 10 h light: dark, 25C). Recovered *Fusarium* isolates were then transferred to PDA and CLA and identified.

Once it was determined that the method worked successfully, several other plant species were inoculated with *F. avenaceum* isolate F5-3 as above, to further examine the host range of *F. avenaceum* (Figure 2).

Isolate & ID number	Source
F. avenaceum (peach) 14-22	ARC, from canola (2000)
F. avenaceum (red) F5-3	ARC, from canola (1999)
F. avenaceum (red) 5	ARC, from canola (2000)
F. graminearum 194192	ECORC, from wheat (1992)
F. oxysporum 213293	ECORC, from corn (1991)

Figure 1. Isolates used in host range inoculation experiments, and their sources.

Crop Species		**Forage Species**		**Weed Species**	
Barley (Harrington)	Mustard (*Sinapis alba*:Viscount (yellow); *B. juncea*: Common Brown)	Alfalfa (Algonquin)	Creeping Red Fescue (Boreal)	Canada Thistle	Mustard: wild, ball, wormseed
Canola (*Brassica napus*: Nexera 705, Quantum, Quest; *B. rapa*: Reward)	Oats (unknown)	Smooth Brome Grass (Carlton)	Orchard Grass (Potomac)	Chickweed	Narrow-leaved Hawk's-beard
Corn (Carousel)	Peas (Patriot)	Red Clover (common seed)	Russian Wild Rye (Mayak)	Cleavers	Pineappleweed
Flax (unknown)	Wheat (Katepwa)	White Dutch Clover (common seed)	Timothy (Climax)	Flixweed	Shepherd's Purse
				Green Foxtail	Perennial Sow-thistle
				Hairy Nightshade	Stinkweed
				Hemp-Nettle	Wild Buckwheat
				Lamb's Quarters	Wild Oats

Figure 2. Plant species tested in the host range experiments.

Pathogen Mode of Action

In a method from Benard and Punja (1995), the *Fusarium* isolates were tested for pectolytic enzyme production, to determine if there was a correlation between pathogenicity and enzyme production. Twelve isolates of *Fusarium* spp. of varying pathogenicity were tested (Figure 3).

Isolate ID	Species	Pathogenicity	Source
F5-3	*F.avenaceum*	Degenerated	ARC 1999
5	*F.avenaceum*	High	ARC 2000
8	*F. oxysporum*	Mod	ARC 2000
9	*F. avenaceum*	Mod	ARC 2000
11	*F. avenaceum*	High	ARC 2000
13	*F. culmorum*	None	ARC 2000
14	*F.avenaceum*	High	ARC 2000
16	*F. oxysporum*	High	ARC 2000
22	*F.oxysporum*	Mod to high	ARC 2000
194176	*F. avenaceum*	High	ECORC
194192	*F. graminearum*	High	ECORC
213293	*F. oxysporum*	High	ECORC

Figure 3. Isolates of *Fusarium* spp. included in the pectolytic enzyme assay.

Cultivar Susceptibility

Laboratory Screening

Attempts were made to develop a simple laboratory method for screening seedlings of *Brassica napus* cultivars for resistance to Fusarium wilt. Seedlings were grown by surface sterilizing seeds of Nexera 705 (known to be highly susceptible) and Quantum (known to have some level of resistance), and growing them on sterile, wet filter paper in Petri dishes in a humidity chamber on a lab bench at ambient temperature (about 22C). Seedlings were inoculated at the cotyledon stage, using inoculum prepared by the same method employed in the pathogenicity experiments. Several methods of inoculating canola seedlings were tested.

Method 1: inoculum was dispensed directly onto roots until filter paper was saturated.

Method 2: roots were lightly wounded by gently cutting with a scalpel, and inoculum was dispensed directly onto roots until filter paper was saturated.

Method 3: inoculum was sprayed onto entire plant.

Method 4: roots were severed with scalpel so approximately 0.5 cm of root remained, and inoculum was dispensed directly onto roots until filter paper was saturated.

Stem inoculation Trial

A total of eleven *Brassica napus, B. rapa* and *B. juncea* cultivars were inoculated with *F. avenaceum* isolate F5-3 using the cotton swab stem inoculation technique described above. The plants were at BBCH growth stage 32-34. The inoculated plants were incubated in a growth chamber set at 20° C/18° C day/night temperature with a 16h diurnal cycle. Five plants were grown per 6-inch pot in Sunshine® soilless medium. Plants were arranged in a completely randomized design after inoculation. *Brassica napus* cv. Quest was included as both inoculated and water-inoculated controls. The swabs and Parafilm were removed three days after inoculation when pink discolouration typical of *Fusarium* spp. was noted in the cotton. Disease severity was evaluated six and eleven days after inoculation by visually estimation the percentage girdling visible on each plant. Disease severity was also determined by measuring stem diameter at the inoculation point 20 days after inoculation. To remove the effects of genotypic and plant-to-plant variation, constriction due to *F. avenaceum* infection was expressed as a percentage of stem diameter at the second internode. After all stem measurements had been made, the plants were cut off at ground level, weighed, and oven-dried at 60° C until dry (approximately 3 days). Dry weight was expressed as a percentage of fresh weight prior to statistical analysis. Girdling estimates and constriction measurements were subjected to arc-sine transformation prior to analysis using PROC GLM of SAS (SAS Institute Inc. 2000).

Field Trial

Seventeen *Brassica napus* cultivars were seeded in a replicated large plot experiment by the CPC at Ranfurly, AB. A natural Fusarium wilt infection developed in the trial, and the cultivars were evaluated for susceptibility to the disease by counting the number of infected plants in the second and third rows of each plot.

Yield Loss Estimates

In order to estimate the potential yield losses attributable to the disease, yield loss estimates were made in an affected field planted as a canola variety strip trial in east-central Alberta. To reduce the confounding effects of other diseases, an area with low levels of The historic and contemporaneous levels of Blackleg, Sclerotinia stem rot, Alternaria black spot and other diseases at the site were low, which minimized the confounding effects of other diseases. In addition, plants exhibiting symptoms of any canola disease other than Fusarium wilt were excluded from the analysis. All canola

plants were removed from four randomly selected 2.5m^2 plots per canola variety. The varieties examined were Nexera 705, which had a high level of disease, and Quantum, which had a low level of disease. The number of severely affected, moderately affected and unaffected plants in each plot was determined. Plants in each of the three severity categories were dried, threshed, and the seed was cleaned and weighed. The amount of seed produced by severely affected and moderately affected plants was compared to plants without the disease. This procedure gave an estimate of yield losses on a per area basis.

Disease Survey

Canola fields in western Canada were surveyed for the presence and incidence of Fusarium wilt. Pathologists and Crop Specialists in Alberta participated in the survey. Ninety fields throughout Alberta were examined: 20 in the north Peace, 30 in the south Peace, 10 in central Alberta, 19 in east central Alberta, and 11 in southern Alberta. One hundred plants were sampled per field, and diseases were rated as percentage of field infected. Surveys began at late flowering (Harker-Berkenkamp growth stage 4.4) and continued through podding (up to Harker-Berkenkamp growth stage 5.3). Plant cultivar, growth stage, weed species/density, crop stand and the presence or absence of other diseases was recorded. Samples of symptomatic plants from some affected fields were brought to ARC-Vegreville for confirmation of diagnosis and for fungal isolation. Saskatchewan and Manitoba were surveyed by Saskatchewan and Manitoba Agriculture and Agriculture Canada staff.

RESULTS AND DISCUSSION

Isolations

Isolation from Stems

Isolations in 1999 produced 12 isolates of *Fusarium* spp. that were purified and maintained for future studies. These were mostly *F. avenaceum* (red and peach coloured isolates) and some *F. oxysporum*.

In 2000, isolations from dried samples of infected canola tissue resulted in far less contamination than fresh-stem isolations, regardless of method. Methods 1 and 2 bore comparable results, although rinsing with sterile distilled water seemed to result in more contamination with both methods. It was decided that method 1, without rinsing, would be used for future isolations. Successful isolations were made from both the lower and mid-stems. Approximately 15 *Fusarium* isolates were purified and maintained for use in further studies.

On PDA, culture morphology included types that were dark rose, peach, cream, and bluish-black, with varying growth rates and differing in amount of aerial mycelium.

Isolation from Seed

In 1999, on average, more Fusarium wilt colonies were obtained from seed from highly infected plants than from plants that were moderately infected or uninfected. Because there was a high variation in colony numbers, results were not statistically significant at the 5% level (Table 1). In 2000, two separate experiments yielded similar results. The first experiment involved using three different methods. Isolations on N-S media showed that 100% of untreated seeds grew various types of fungi. Isolations using method 2 showed that 20% of seeds from uninfected canola grew various fungi, versus 47% of seeds from the infected canola. Isolations using method 3 showed that 0% of seeds from uninfected canola grew fungi, versus 27% of seeds from the infected canola. There were no differences in the types of fungi isolated among treatments or isolation methods. A second experiment in 2000, using method 3, also showed that seed from infected plants tended to produce more fungal colonies (Table 2). These results concur with those of Clear and Patrick (1995), who found that of all the *Fusarium* species, *F. avenaceum* was most frequently isolated from surface-disinfested canola seed. While not definitive, these results do suggest that Fusarium wilt can be seed-borne. A more detailed study of the transmission and virulence of *F. avenaceum* isolated from seed is required.

Table 1. Analysis of variance of *Fusarium* spp. colony formation by seed from highly infected, moderately infected, and uninfected Nexera 705 canola plants in 1999.

Treatment	No. of *Fusarium* Colonies	No. of seeds plated	% Infected seed[1]
Severely Infected	30	76	40a
Moderately Infected	35	200	18a
Uninfected	30	195	15a

[1] Means with the same letter are not significantly different at the 5% level.

Table 2. Fungal colonization of infected and uninfected seed of two *Brassica napus* of cultivars in 2000.

Treatment	Percent of seeds forming fungal colonies
Nexera 705-heavy infection	46a[1]
Quantum-infected	20b
Nexera 705-light infection	8b
Nexera 705-uninfected	4b
Quantum-uninfected	12b

[1] Means with the same letter are not significantly different according to a Duncan's multiple range test performed at the 5% level.

Pathogenicity Experiments

Root dip pathogenicity test

1999

Re-inoculation of *Brassica napus* plants with *Fusarium avenaceum* isolates obtained from wilted plants produced severe wilt symptoms, whereas re-inoculation with *F. oxysporum* isolated from wilted plants resulted in only slight wilt symptom development. As Table 3 shows, *F. avenaceum* isolates F5-3 and F2 killed 80% to 95% of the inoculated plants, and were signifcantly more virulent (ANOVA P>F=0.0363) than *F. oxysporum* (isolates Krahn and F1), which killed 40% of the plants. Symptoms produced by *F. avenaceum* inoculation were more severe than those observed in farm fields; this was most likely caused by the severe nature of the inoculation method.

Table 3. Virulence of seven single-spore *Fusarium* spp. isolates obtained from wilted canola plants on *Brassica napus* cv. Quest plants inoculated using a root-dip method.

Isolate number	*Fusarium* species	Isolate origin	Surviving non-wilted plants, 42 days post-inoculation[1]
Quest 857	*oxysporum*	Vegreville	4.75a[2]
Non-inoculated Control	-	-	4.75a
F3	*oxysporum*	Vegreville	3.75ab
Krahn	*oxysporum*	Vermilion	3.00b
F1[3]	*oxysporum*	Ft. Vermilion	3.00b
F4	*oxysporum*	Vegreville	2.75b
F5-3[3]	*avenaceum*	Ft. Vermilion	1.00c
F2[3]	*avenaceum*	Ft. Vermilion	0.25c

[1]Number of surviving plants per pot. Five plants were transferred to each pot after inoculation. N=32.

[2]Means in a column with the same letter are not significantly different according to a Duncan's multiple range test conducted at the 5% level.

[3]Identified to species by Randy Clear, Canadian Grain Commission, Winnipeg, MB.

2000

In 2000, several of the isolates *Fusarium avenaceum* isolated from symptomatic plants collected in the field were pathogenic when re-inoculated to canola plants (Table 4). Severe wilt symptoms resulted in the deaths of approximately 60% to 100% of the plants whose roots were inoculated with *F. avenaceum*, irrespective of leaf stage.

Fusarium avenaceum was re-isolated from the roots, lower stems, and upper stems of the inoculated canola plants that showed signs of pathogenicity. These pathogenicity experiments met the requirements of Koch's postulates:

1. The agent must be present in every case of the disease.
2. The agent must be isolated from the host and grown in pure culture.
3. The disease must be reproduced when a pure culture of the agent is inoculated into a healthy susceptible host (Figure 4).
4. The same agent must be recovered again from the experimentally infected host.

Fusarium avenaceum was isolated from many diseased canola plants from multiple locations, and upon inoculation into healthy canola plants, caused symptoms not unlike those seen in the field. The symptoms were not identical, but it is not unusual to see symptoms in the laboratory differ somewhat from those seen in the field. Furthermore, we are presently unaware of when, how, and under what conditions infection naturally occurs, which could further result in symptom differences.

Table 4. Pathogenicity ratings on Nexera 705 canola plants inoculated with *Fusarium* cultures obtained from wilted canola plants in the field in 2000.

		Average pathogenicity rating			
		One week post-inoculation		Two weeks post-inoculation	
Isolate	Species	Least-square mean	Standard Error	Least-square mean	Standard Error
F#14-2000	*F. avenaceum*	5.0a	0.2	5.0a	0.4
F#11-2000	*F. avenaceum*	4.8a	0.2	5.0a	0.4
F#5-2000	*F. avenaceum*	4.7a	0.2	4.5a	0.4
F#22-2000	*F.oxysporum*	4.7a	0.2	4.0a	0.4
F#8-2000	*F.oxysporum*	3.3bc	0.2	3.4bc	0.4
F#9-2000	*F.avenaceum*	3.2bc	0.2	3.0bc	0.5
Control[2]	None	2.1c	0.2	0.8c	0.5

[1]Means in a column with the same letter are not significantly different according to a Tukey-Kramer multiple comparison test conducted at the 1% level.

[2]Stress due to root clipping resulted in shock to the plant during the first week post-inoculation; some, but not total, recovery occurred by the second week.

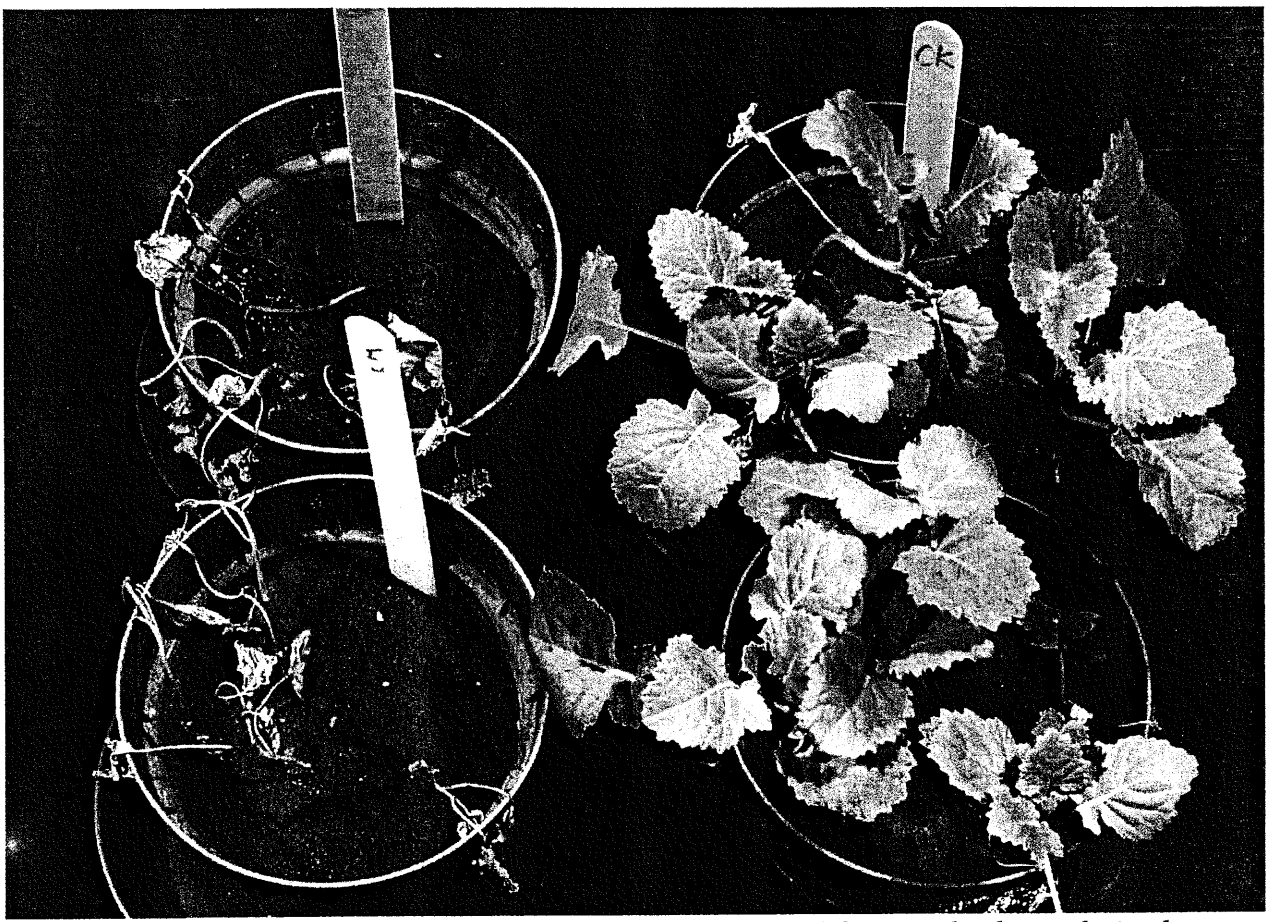

Figure 4. Canola plants inoculated with *F. avenaceum* isolated from wilted canola in the field (left) vs. non-inoculated control (right).

Inoculation stage

F. avenaceum caused wilt symptoms to all growth stages of *Brassica napus* cv. Quest tested, although the severity of symptoms was affected by the stage at which the plants were inoculated. Figure 5, part A shows that the plants inoculated at intermediate growth stages developed less severe symptoms than either very young or more mature plants. Plants that are infected in these intermediate growth stages may be able to compensate for damage caused by *F. avenaceum* by replacing damaged tissues. Very small plants may be unable to do this because of their limited size, or because primary tissues may be more susceptible to infection than periderm or other secondary tissues. More mature plants may be unable to compensate for damage because tissues have already differentiated into reproductive or senescent forms and cannot quickly replace lost vegetative parts.

The *F. oxysporum* isolate included in this experiment was less virulent than the *F. avenaceum* isolates. Disease severity was lower, and leaf count, plant height and plant biomass were all less severely restricted by *F. oxysporum* than by *F. avenaceum* (Figure 5). Infected plants in the inter plants tested at the 1- to 7-leaf stages were equally susceptible to *F. avenaceum*.

Stem inoculation

In stem inoculation tests, *F. avenaceum* caused severe wilting (Figure 6) and localized necrosis around the inoculation sites when either the mycelium injection or petiole methods were used (Figure 7); injection of macroconidia into the stem was ineffective (Table 5). The wilting observed in this experiment more closely resembled the symptoms observed in affected farm fields than the plants inoculated using the root dip method.

As seen in the root-dip inoculation tests, *F. avenaceum* was more virulent to canola than *F. oxysporum*. The percentage of stem girdling, averaged across inoculation methods on the two *F. avenaceum* isolates was 55.2% and 36.9%for *F. avenaceum* isolates F5-3 and F2, respectively, while that of *F. oxysporum* isolate F1 was 14.0%. Isolate F5-3 was used in a subsequent cultivar screening test because it was generally more virulent than isolate F2 (Table 5).

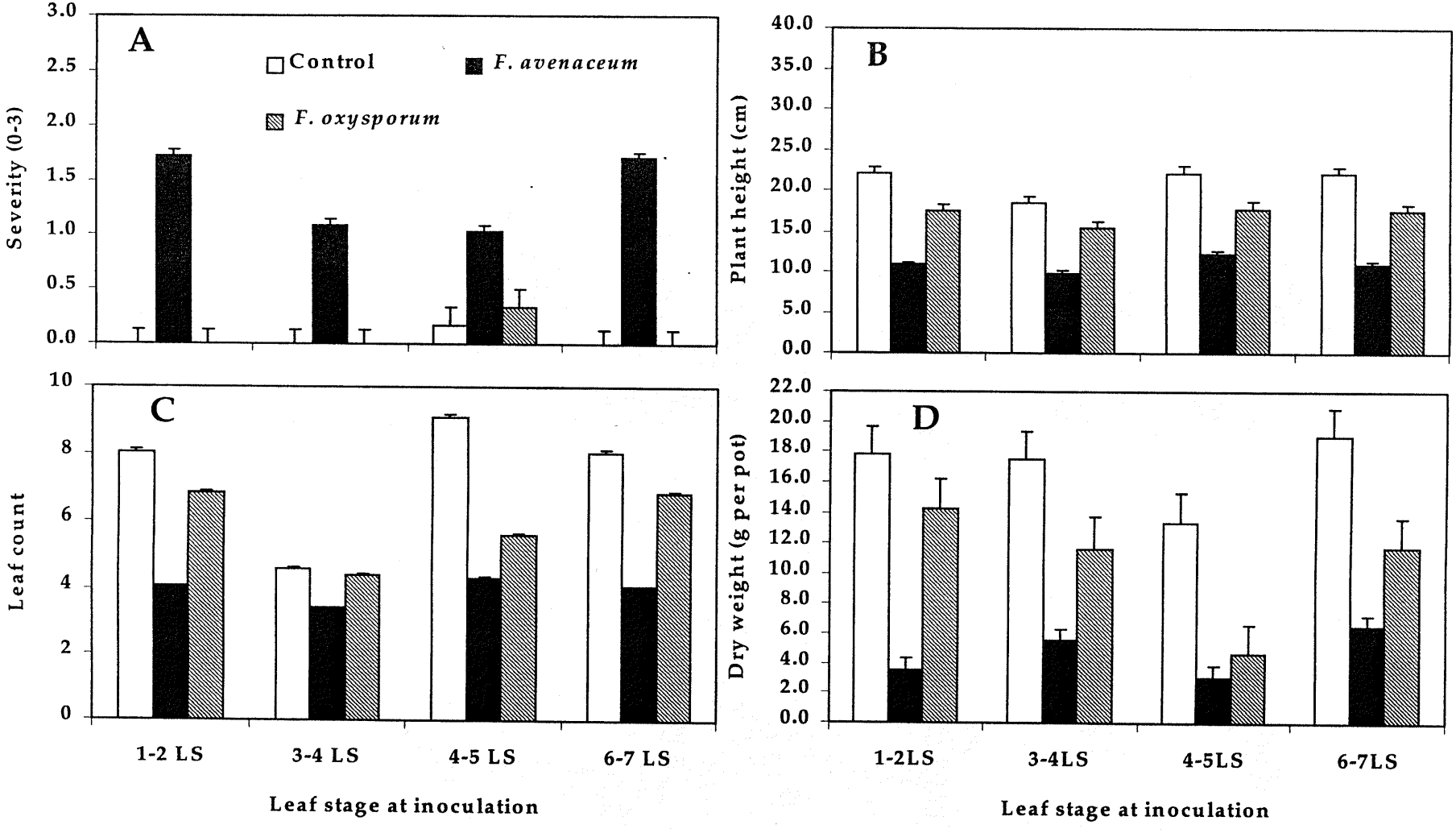

Figure 5. Effect of *Brassica napus* cv. Quest growth stage on severity of wilt symptoms when inoculated with *Fusarium avenaceum* and *F. oxysporum* isolates obtained from symptomatic plants. Each bar is the least square mean of five plants in each of four replicate pots, with data from each *Fusarium* species pooled. Vertical lines indicate standard error of the least square mean

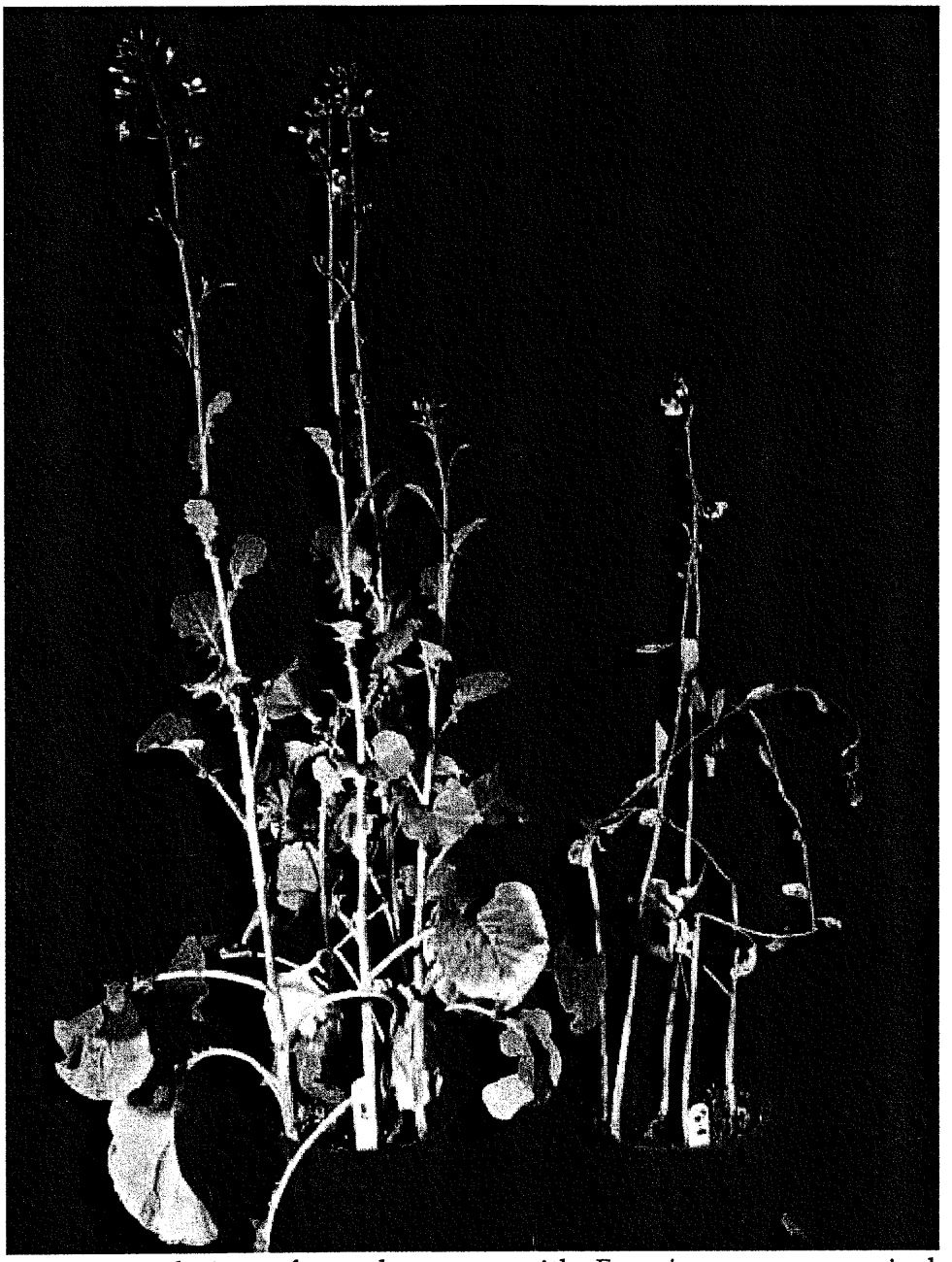

Figure 6. Inoculation of canola stems with *Fusarium avenaceum* isolate F5-3 caused generalized wilt symptoms (right). Non-inoculated control plants are pictured on the left.

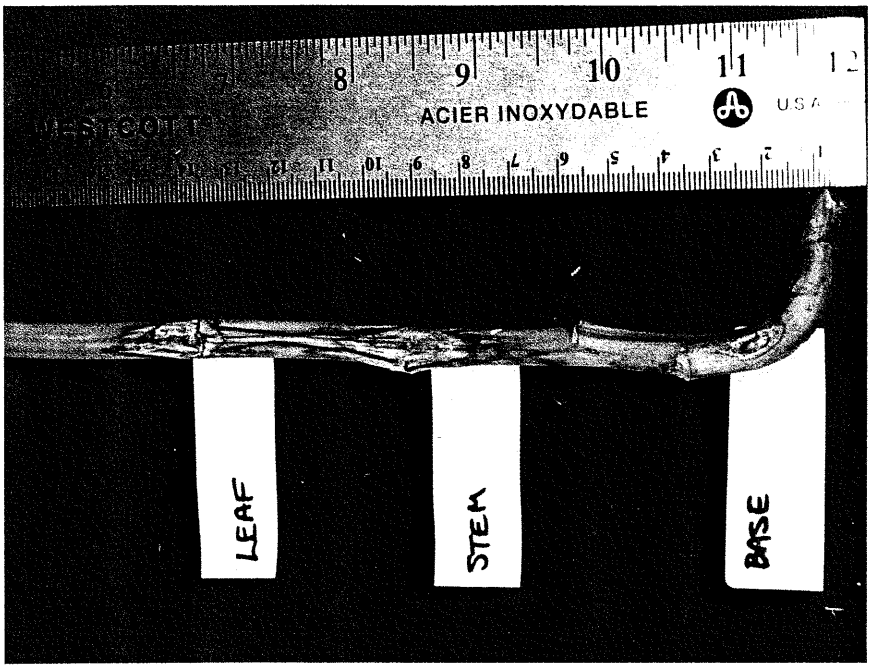

Figure 7. Comparison of three stem inoculation techniques. The canola stem was inoculated with *F. avenaceum* by applying suspended macroconidia in a cotton swab (left; "LEAF"), by inserting fungal mycelium into a wound made in the stem (center; "STEM"), and by injecting 100µl of conidiospore suspension into the stem (right; "BASE").

Table 5. Virulence of three *Fusarium* spp. isolates on *Brassica napus* cv. Quest inoculated using three stem inoculation techniques.

Inoculation method	Isolate	*Fusarium* species	Girdling %
Petiole inoculation	F5-3	*avenaceum*	91.0a[1]
	F2	*avenaceum*	41.4cd
	F1	*oxysporum*	5.0ef
	Control		0.0f
Mycelium insertion	F5-3	*avenaceum*	67.5b
	F2	*avenaceum*	61.3bc
	F1	*oxysporum*	27.4de
	Control		0.8f
Spore injection	F1	*oxysporum*	9.5ef
	F2	*avenaceum*	8.1ef
	F5-3	*avenaceum*	7.0ef
	Control		0.0f

[1]Least-square means in a column with the same letter are not significantly different according to a Tukey's multiple range test conducted at the 5% level. N=232.

Seed Germination Tests

Germination tests in 1999 and in 2000 on water agar showed no statistically significant differences in germination among seed from highly infected, moderately infected, and uninfected canola plants, although there was a trend indicating that seed from infected plants had lower germination rates. Germination tests in 2000 in soil-less mix, however, showed significantly lower germination of seed from heavily infected Nexera 705 plants (Table 6). These results suggest that seed harvested from Fusarium wilt-infected canola may have lower germination rates than seed from uninfected canola.

Table 6. Comparison of percent germination in soil-less mix of seeds obtained from infected versus uninfected plants of two canola varieties in 2000.

Treatment	Percent germination
Nexera 705-heavy infection	48a[1]
Quantum-infected	66b
Nexera 705-light infection	70b
Nexera 705-uninfected	78b
Quantum-uninfected	69b

[1] Values with the same letter are not significantly different at the 5% level according to Duncan's multiple range comparison.

Host Range Experiments

A range of interactions resulted when *F. avenaceum* originally isolated from wilted canola was used to re-inoculate a broad range of crop and weed species. Reaction of plants to infection ranged from those showing no symptoms to several exhibiting various degrees of wilting and death (Table 7, Figure 8). *F. avenaceum* could be re-isolated from all of the crop and weed species tested, even from asymptomatic plant species. Furthermore, successful re-isolation was achieved from both the roots and the stems of all the plant species tested. This suggests that a large number of plant species can act as hosts to the pathogen, and that barley, smooth brome, wheat and white Dutch clover can apparently serve as symptomless carriers of *F. avenaceum*. This is not entirely surprising, as *F. avenaceum* has been shown to have a host range in excess of 150 plant genera (Booth and Waterston 1964).

Our results suggest that crop rotation may have limited potential as a control method of Fusarium wilt of canola since there is abundant availability of possible hosts to allow the propagation and spread of *F. avenaceum*. The fact that it has already been found in diverse areas of the province also supports this notion.

Table 7. Susceptibility of selected plant species to inoculation with various *Fusarium* spp.

Isolate species and ID #	Highly susceptible plant species	Moderately susceptible species	Asymptomatic plant species
F. avenaceum (peach) #14-22	Canola, Flax, Oats	Chickweed, Peas, Wild Oats (WO)	Alfalfa, Barley, Corn, Red Clover (RC), Smooth Brome Grass (SBG), Wheat, White Dutch Clover (WDC)
F. avenaceum (red) #F5-3	Alfalfa, Canola, Flax, Oats	Chickweed, Corn, Peas, RC, WO	Barley, SBG, Wheat, WDC
F. avenaceum (red) #5	Alfalfa, Canola, Flax, Oats	Chickweed, Corn, Peas, RC, WO	Barley, SBG, Wheat, WDC
F. graminearum #194192	Corn, Flax, Oats, RC, Wheat, WO	Alfalfa, Chickweed	Barley, Canola, Peas, SBG, WDC
F. oxysporum #213293	Flax	Canola, Peas, Chickweed, WO	Alfalfa, Barley, Corn, Oats, RC, SBG, Wheat, WDC

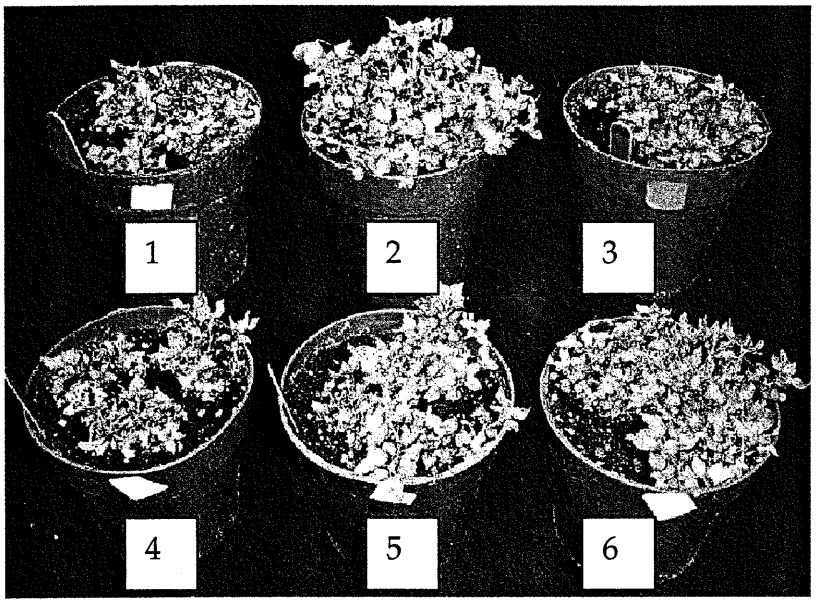

Figure 8. Result of the host range study on chickweed, a plant species that was found to be moderately susceptible to *Fusarium* spp. Plants in pot 1 were inoculated with *F. avenaceum* isolate 14-22, pot 2 was the uninoculated control, pot 3 was a positive control, inoculated with *F. graminearum* isolate 194192, pot 4 was inoculated with *F. avenaceum* isolate 5, pot 5 was inoculated with *F. avenaceum* isolate F5-3, and pot 6 was a positive control, inoculated with *F. oxysporum* isolate 213293.

Pathogen Mode of Action

All the *Fusarium* isolates produced pectolytic enzymes to some extent. In general, the pathogenic isolates produced more enzyme activity per mg of mycelium than non-pathogenic isolates (data not shown). There were some exceptions: a previously highly pathogenic isolate degenerated to the pionnotal form, and pectolytic enzyme production was very low. Cultural degeneration, resulting in avirulence, is a major problem in studies on *Fusarium* (Burgess et al. 1994). Though pectolytic enzymes may be involved in the pathogen mode of action, at this point, no conclusions can be drawn.

Cultivar Susceptibility

Laboratory Screening

A cultivar screening method, similar to that for Blackleg, would be useful to plant breeders for selecting new canola lines. Although the method described in the pathogenicity experiments section could be used to screen cultivars, it is too time consuming and labour-intensive to be useful in the type of mass line screening required by breeders.

The four methods tried all resulted in significant levels of disease in both cultivars tested. However, the differences between the cultivars is likely not large enough or consistent enough to make any of these methods a useful screening tool. Unfortunately, little field data is available to know the levels of susceptibility or resistance in other cultivars, so it is currently difficult to extrapolate the results of any laboratory trial to what occurs in the field. Development of a simple screening tool that could differentiate between susceptible, moderately susceptible, moderately resistant, and resistant cultivars of canola would be very useful.

Stem inoculation

This experiment was designed to determine if differential reactions to *F. avenaceum* infection could be induced by stem inoculation. *Brassica* genotypes were chosen on the basis of previously observed reactions to the disease in field trials and surveys. Quest was chosen because it was frequently observed to be severely affected in field surveys. Plants grown from seed harvested from the Andrew, AB site where Fusarium wilt was first documented was also included as an additional susceptible control. The genotypes 3GL-08, 3GL-23 and 5KS-215 were severely and reproducibly affected by wilt in a blackleg nursery near Lavoy, AB. Midas was chosen because is an older cultivar which may differ from current cultivars in susceptiblity to wilt. *Brassica rapa* and *B. juncea* were included to determine if these species were more susceptible than *B. napus*. Stem inoculation produced wilted plants, although necrosis and constriction of the stem near the inoculation site were also observed. Cultivars could not be distinguished using percent girdling as a criterion (Table 8), as *F. avenaceum* affected all treatments equally, except for the non-inoculated control. Percent constriction at the wound site was not different between the inoculated and non-inoculated treatments, indicating the observed damage at the inoculation site was associated with the inoculation method and not with *F. avenaceum* infection.

Table 8. Least-square mean severity of symptoms on nine *Brassica* spp. cultivars after stem inoculation with *Fusarium avenaceum*.

Treatment	*Brassica* species	Percent girdling at inoculation site[1] 6 DPI	11 DPI	Severity increase	Percent contriction 20 DPI[1]	Percent dry weight 20 DPI
Midas	*napus*	-	-	-	-	75.2a
Common Brown	*juncea*	9.1a[2]	9.7a	0.6ab	23.8a	66.0ab
Westar	*napus*	8.4a	9.1a	1.3ab	30.6a	35.8bc
Tobin	*rapa*	9.0a	9.1a	0.7ab	11.9a	35.2bc
3GL-08	*napus*	8.3a	9.4a	1.2ab	19.1a	35.1bc
3GL-23	*napus*	9.0a	8.9a	0.6ab	25.2a	27.7c
5KS-215	*napus*	9.1a	9.4a	0.5ab	9.9a	22.8c
Quest	*napus*	8.4a	8.7a	0.7ab	8.6a	18.2c
Goldrush	*rapa*	9.0a	9.0a	0.6ab	24.1a	17.9c
Andrew Quest	*napus*	8.1a	8.8a	1.6a	20.1a	17.9c
Quest (uninoculated)	*napus*	0.0b	0.0b	0.0b	26.4a	16.8c

[1]Data were arc-sine transformed ($arcsine((x+0.5)\ 100^{-1})$ prior to analysis. Back-transformed values are presented here.

[2]Least-squre means in column with the same letter are not different according to a Tukey's multiple range test conducted at the 5% level.

Field Trial

Incidence of Fusarium wilt was quantified in each of the seventeen *Brassica napus* cultivars in the CPC trial at Ranfurly, AB. Incidence in LG 3525 was significantly higher than in LL2631. The remaining cultivars were not significantly affected by Fusarium wilt (Figure 9).

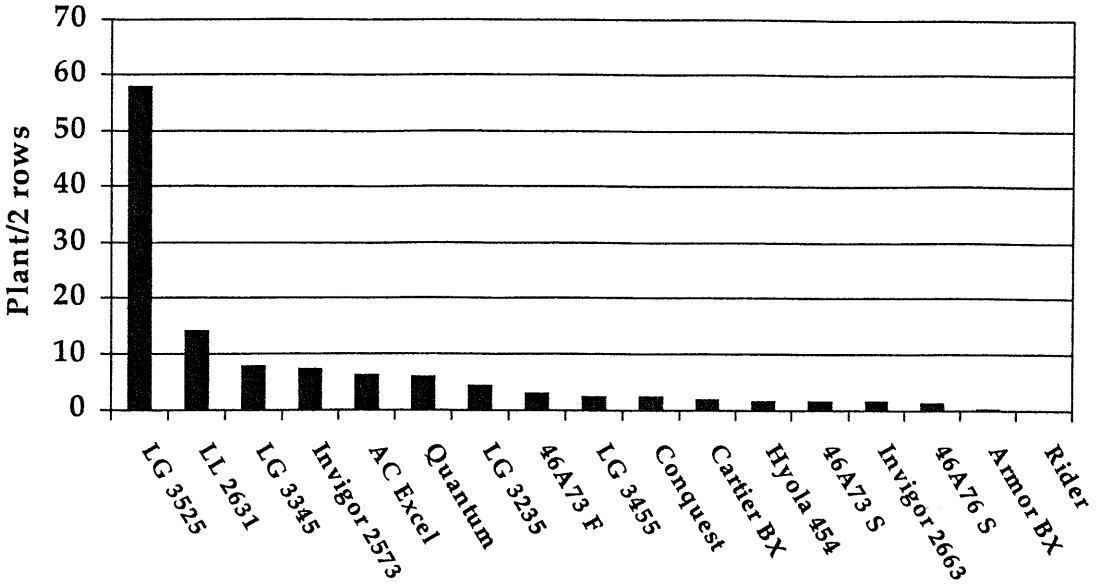

Figure 9. Incidence of Fusarium wilt in seventeen *Brassica napus* cultivars at Ranfurly AB in 2000.

Yield Loss Estimates

Results of the yield loss estimate experiment indicate that Fusarium wilt may substantially decrease seed yield, and may reduce crop stand (Table 9).

Table 9. Disease severity, incidence, plant stand count, and yield differences between two canola varieties infected with Fusarium wilt.

Variable	Nexera 705 (Susceptible)	Quantum (Resistant)
Mean Disease Severity	0.47*	0.01*
Mean Disease Incidence	37.5 plants/ m^2	1.0 plants/ m^2
Mean Plant Stand	45 plants/ m^2	73 plants/ m^2
Mean Estimated Yield	**0.9 tonnes/ ha**	**1.5 tonnes/ ha**

* Scale used is 0 – 2, where 0 = no disease, 1 = light disease, and 2 = severe disease.
Note that all differences between means are statistically significant according to T-tests performed at the 1% level for all variables.

Disease Survey

Results from the 2000 canola disease survey focused on four diseases, including Fusarium wilt (Table 10). The results indicate that Fusarium wilt is concentrated in east-central Alberta, with some occurrences in the north Peace Region. Overall, the disease is not as serious as well-established diseases such as Blackleg or Sclerotinia stem rot;

however, Fusarium wilt incidence exceeded 20% in some individual fields, and reached 72% in one location near Sedgewick. One occurrence was confirmed near Carman Manitoba. Reports of Fusarium wilt in the BC Peace and near North Battleford, SK were also received, but were not confirmed.

Table 10. Canola diseases in Alberta in 2000.

		% Disease Incidence				
		Sclerotinia[2]				
Region[1]		Main stem	Upper/ pod	*Blackleg*	**Fusarium wilt**	Black spot severity[4]
North Peace	20	8.7	4.6	T[3]	**0.8**	0.2
South Peace	30	6.3	1.9	0.4	**0.0**	T
Central	10	8.2	7.2	1.6	**0.6**	0.3
East Central	19	11.0	14.0	22.0	**8.3**	1.3
South	11	0.0	0.0	2.1	**0.0**	T
Overall	90	7.3	5.4	5.6	**2.0**	0.4

[1] The regions surveyed included the following cities and towns:
North Peace = Fairview, Grimshaw, Manning, Fort Vermilion, La Crete
South Peace = Beaverlodge, Falher, La Glace, Nampa, Spirit River, Wanham, Eaglesham
Central = Leduc, Stony Plain
East Central = Bonnyville, Sedgewick, Vegreville
South = Vulcan, Medicine Hat

[2] Sclerotinia stem rot lesions were scored either as a main stem lesion or as an upper stem/pod lesion.

[3] T =. Trace amounts of disease (<0.1%) or disease was not found in the 100 plant samples but was noted in the field. Trace values were considered as 0.1% for calculating means.

[4] Percent pod area affected by black spot.

CONCLUSIONS AND PROJECT ASSESSMENT

Fusarium avenaceum is the causal agent of Fusarium wilt on canola in the Canadian prairies. Fusarium wilt presently occurs primarily in the Peace region and in the north eastern part of Alberta, but there are also reports from other areas. *Fusarium avenaceum* has a wide host range, including several crop, forage and weed species, and is capable of causing disease symptoms on several of its hosts. Isolations from seed indicate it has the potential to be seed-borne. The occurrence of the disease results in a significant yield loss to the canola grower. The pathogen mode of action may involve the production of pectolytic enzymes. *Brassica napus* cultivars appear to differ in their susceptibility to Fusarium wilt. This is an encouraging result, since it suggests that heritable resistance to Fusarium wilt is already available within *Brassica napus*, obviating the need to introgress resistance genes from exotic sources.

The development of a suitable screening method for evaluation of cultivars by breeders is the logical next step for future research of this disease. The screening method should be a relatively simple laboratory technique that accurately reflects how canola cultivars respond to the disease in the field. Research into the environmental conditions that influence disease development, as well as the transmission of *F. avenaceum* by seed is also required.

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