

## Dynamics of *Rhynchosporium secalis* pathotypes in relation to barley cultivar resistance

Kegnan XI<sup>1\*</sup>, Thomas TURKINGTON<sup>2</sup>, Jon MEADUS<sup>2</sup>, James HELM<sup>3</sup> and Jalpa TEWARI<sup>4</sup>

<sup>1</sup> Alberta Agriculture, Field Crop Development Centre, 6000 C & E Trail, Lacombe, Alberta, T4L 1W1, Canada.

<sup>2</sup> Agriculture & Agri-Food Canada, Lacombe Research Centre, 6000 C & E Trail, Lacombe, Alberta, T4L 1W1, Canada.

<sup>3</sup> Alberta Agriculture, Field Crop Development Centre, 5030 50 Street, Lacombe, Alberta, T4L 1W8, Canada.

<sup>4</sup> Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, T6G 2P5, Canada.

E-mail: kequan.xi@gov.ab.ca

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*Rhynchosporium secalis* isolates E97-2 and H97-2, represented the major pathotypes in populations on barley in Alberta, Canada, but differed widely in their virulence. Following greenhouse co-inoculation with the two pathotypes, E97-2, originally isolated from resistant cv. 'CDC Earl', predominated over H97-2, isolated from the susceptible cv. 'Harrington', from the first to the last of four infection cycles on both 'CDC Earl' and 'Harrington'. These results indicated that the host can rapidly influence pathotype composition and that pathotype E97-2 may have a competitive advantage over H97-2 on these cultivars. DNA polymorphisms were found between isolates from single or mixed inoculations on cvs 'CDC Earl' and 'Harrington' for four successive cycles. Co-inoculation with the two isolates resulted in a shift to a molecular phenotype more similar to E97-2 than H97-2. The competitive advantage of E97-2 over H97-2, combined with the selective pressure exerted by the host, would explain the increased susceptibility of cv. 'CDC Earl' and other cultivars with similar sources of scald resistance, in fields across Alberta. However, H97-2 will likely remain one of the major pathotypes in Alberta due to the relatively large acreage of cv. 'Harrington' in this province.

### INTRODUCTION

Changes in the frequency of virulence in the barley scald pathogen *Rhynchosporium secalis* are frequently directly related to changes in the genetic composition of the host. Such changes have led to the increasing prevalence of pathotypes virulent on widely grown and formerly resistant cultivars. The breakdown of resistance of the barley cv. 'Atlas 46' (*Hordeum vulgare*) in California was documented as a result of virulence variability in the scald pathogen (Jackson & Webster 1976a). Jackson & Webster (1976b) found that when a mixture of five races of widely varying pathogenicity was inoculated and carried through two infection cycles on the host and an intervening saprophytic stage, variability in virulence resulted from the generation of new races. A positive correlation between the virulence in several *R. secalis* races and the resistance of barley composite crosses has been demonstrated (Jackson *et al.* 1982, Zhang, Webster & Allard 1987).

A high level of variability in the virulence of the scald pathogen in western Canada has also been documented

(Tekauz 1991, Xi *et al.* 2002a). Pathotypes E97-2 and H97-2 of *R. secalis* originally isolated from cvs 'CDC Earl' and 'Harrington', respectively, showed differences in virulence spectra and were found to be among the most predominant pathotypes in Alberta (Xi *et al.* 2002a). Scald has been a major barley disease in Alberta and the use of resistant cultivars is one of the main methods for scald management (Turkington *et al.* 1998). Over the past several years it has been observed that the previously scald-resistant cultivars 'CDC Earl', 'CDC Guardian' and 'Duke' have had high levels of scald infection in experimental plots and commercial fields across Alberta (Turkington *et al.* 1998, 1999, Xi *et al.* 2000). It was suggested that the resistance in these cultivars was due to a single or a few major genes (Penner *et al.* 1996). However, it is unclear how the resistance in these barley cultivars affected the composition and frequency of major *R. secalis* pathotypes in Alberta. The prevalence of virulent races may also be the consequence of the relative survival and competitive abilities of one pathogen race over others on particular host cultivars (Thurston 1961). These abilities can be partitioned into components including over-wintering in a saprophytic phase, and the processes of inoculum

\* Corresponding author.

discharge, germination, penetration, colonization and sporulation. Interactions among various components of the life cycle of a pathogen and competition with other pathogen races may contribute to shifts in race composition, thereby changing the virulence patterns in a pathogen population.

The objective of the present study, therefore, was to test the hypothesis that shifts in *R. secalis* pathotype composition result from the selection pressure exerted by resistant genotypes in the host population. Barley hosts differing in resistance were used to determine the relative competitive and adaptive ability of pathotypes of *R. secalis* in inoculum mixtures. The random amplified polymorphic DNA (RAPD) assay was used to fingerprint the parent and off-spring pathotypes from inoculation mixtures. The resulting molecular phenotypes were then compared with pathotype virulence identified using the host cultivars.

## MATERIALS AND METHODS

### *Pathotype evaluation*

*Rhynchosporium secalis* isolates, E97-2 and H97-2, were obtained from scald lesions on leaves produced from natural infections on cvs 'CDC Earl' and 'Harrington', respectively. The two isolates were identical by spore morphology and colony colour, but were differentiated on barley cultivars that had been classified into several groups based on scald reaction (Penner *et al.* 1996); E97-2 was virulent on cvs 'Harrington', 'CDC Earl', 'CDC Guardian', 'CDC Richard', 'Duke', 'Turk' and 'Winchester', whereas H97-2 was virulent on 'Harrington' and 'Manley'. Furthermore, E97-2 was found to sporulate more than H97-2 both *in vivo* and *in vitro*. Complex pathotypes are defined as those that possess relatively more virulence genes and are virulent on more genotypes of the host compared with simple pathotypes (Webster, Saghai-Marouf & Allard 1986). As a consequence, E97-2 was considered to be a complex pathotype and H97-2 a simple one.

Sporulation *in vitro* of each pathotype was determined by inoculating eight lima bean agar (LBA) medium plates, with approximately  $1 \times 10^5$  conidia onto each plate. The cultures were incubated at 15–20 °C for 14 d before spore production was assessed using previously described procedure (Xi *et al.* 2000a). Sporulation *in vivo* was determined using procedures described by Xi *et al.* (2000). To determine if spore viability affected isolation frequency, conidia of the two pathotypes were used to test spore germination. Conidia were washed off from two-week old cultures grown on LBA medium, with spore concentrations adjusted to  $5 \times 10^4$  conidia ml<sup>-1</sup>. Approximately 10 drops of barley leaf sap was added to the spore suspensions to stimulate germination. The suspensions were kept at 20 ° for 48 h before percentage spore germination was assessed.

To determine if shifts in pathogen virulence can occur, single-spore cultures of the two *R. secalis* isolates were each inoculated onto the host cultivars from which they had been isolated. The fungus was then isolated from infected leaf material on LBA using the procedures described previously by Xi *et al.* (2002a). Spore suspensions derived from single-spore isolates were inoculated back onto cvs 'Harrington' and 'CDC Earl'. The procedures of inoculation, isolation and re-inoculation were repeated four times, using 5–10 single-spore cultures per isolate each time. Spore suspensions of the two isolates also were mixed in equal proportions and inoculated onto seedlings of cvs 'Harrington' and 'CDC Earl'. Manley was inoculated as a differential to aid in the identification of pathogenic and molecular phenotypes. The fungus was re-isolated from infected leaf material of cvs 'Harrington' and 'CDC Earl', and single-spore cultures were back inoculated to the host cultivars. Adjusted spore concentrations were the same for both isolates and ranged between  $2\text{--}8 \times 10^5$  conidia ml<sup>-1</sup>, depending on spore production in each replicated test. The detailed inoculation and incubation procedures were similar to those described by Xi *et al.* (2002a). The inoculation and incubation procedures were carried out four times resulting in four cycles, using single-spore isolates from 20 to 40 colonies for each pathotype at each time. To prevent cross-contamination, the same pathotype was arranged in one misting chamber as much as possible for inoculation, while plastic sheets were used to separate pots of plants when many pots with different pathotypes were kept in the same chamber. Plants were watered only from the base 2–3 times weekly to avoid splash dispersal that would have been the major source of contamination during the experiment. In each test, a *R. secalis* check isolate was used to test for the repeatability of disease reactions. The experiment consisting of four cycles of inoculation, incubation, and isolation was repeated four times for the single isolates and mixture.

Disease assessments were made on leaves 2 and 3, 14 d after inoculation using the 0–3 reaction scale described by Jorgensen & Smedegaard-Petersen (1995). An average reaction rating equal to or above 1.4 on one of the cultivars was considered to be a virulent pathotype, whereas ratings below 1.4 indicated avirulence. This rating was found to adequately differentiate the relative frequency of individual pathotypes (Xi *et al.* 2002a). An isolate that resulted in a rating below 1.4 on all cultivars was considered to be avirulent. At the time of disease assessments, no lesions were apparent on those leaves that had not yet emerged at the time of inoculation, indicating that there had been no movement of the pathogen between or within plants and leaves under the experimental conditions.

### *Determination of molecular phenotype*

Arbitrary decamer primer Kits A and X were obtained from Operon Technologies (Alameda, CA) and

50 primers of 'Set 1' from the University of British Columbia biotechnology laboratories (Vancouver). All primers initially were used for amplification of major polymorphic bands in isolates E97-2 and H97-2. After a minimum of two runs for repeatable polymorphism, ten primers, OPA-20, OPX-3, 5, 7, and UBC-6, 8, 9, 16, 23 and 25 were then used to amplify *R. secalis* isolates from single and mixed inoculations.

Mycelia and conidia of two-week-old cultures were scraped from the cultures on LBA medium and ground to a fine powder in liquid nitrogen using a pre-cooled pestle and mortar. Powdered genomic DNA material of each isolate was extracted by the SDS miniprep method of Raeder & Broda (1985). The pellet of DNA was washed with 70% cold ethanol, dried and dissolved in 50 µl TE buffer. DNA concentrations were estimated by absorbance at 260 and 280 nm. All DNA samples were stored at  $-20^{\circ}$  until further use.

Polymerase chain reaction (PCR) amplifications were performed in a 25 µl volume containing about 25 ng of genomic DNA, 1–1.5 units of Taq DNA polymerase (Sigma, Aldrich, Oakville, ON), 50 pmole of each primer and dNTP mix (0.2 mM each of dCTP, dGTP, dATP and dTTP) in a reaction buffer with a final concentration of 20 mM Tris-HCl at pH 8.4, 50 mM KCl, and 2.5 mM MgCl<sub>2</sub>. Amplification was performed in a PTC-200 (MJ Research, Watertown, MA) thermal cycler at the following temperature profile: denaturation of DNA at 94 ° for 5 min; primer annealing at 35 ° for 1 min and extension of primer at 72 ° for 1 min. Forty cycles were run at 95 ° for 1 min, 35 ° for 1 min, and 72 ° for 2 min. Subsequently, at the end of the cycles, an additional cycle of 72 ° for 5 min was run. The PCR products of 8 µl were loaded on 1% agarose gels stained with 10 µg/ml ethidium bromide. The products were then analysed by gel electrophoresis in 0.5 × Tris-Borate-EDTA buffer at 100 V for 5 min; 20 V for 15 min and 80 V for 160–180 min, and a 1 kb molecular weight ladder (Invitrogen, Burlington, ON) was used to compare sample DNA fragments. The gel was illuminated on a 320 nm uv light box and photographed with a digital camera. RAPD reproducibility was confirmed by repeating the reactions at least once and using control reactions including standard isolates and a blank.

#### Data analysis

Pathotype frequency on the inoculated cultivars and its interaction with the host over four cycles were examined using the MANOVA procedure (SAS Statistical Software 1989) with repeated-measures analysis of variance and the univariate analysis of variance (Freund, Litted & Spector 1986). The test was repeated four times and each repetition was considered a block. Analyses were done based on a factorial experiment with four blocks. An arcsine transformation was applied to equalize the variance of the frequency data. A sphericity test was used to determine if a univariate

test was valid for the data with a multivariate data structure (Freund, Litted & Spector 1986).

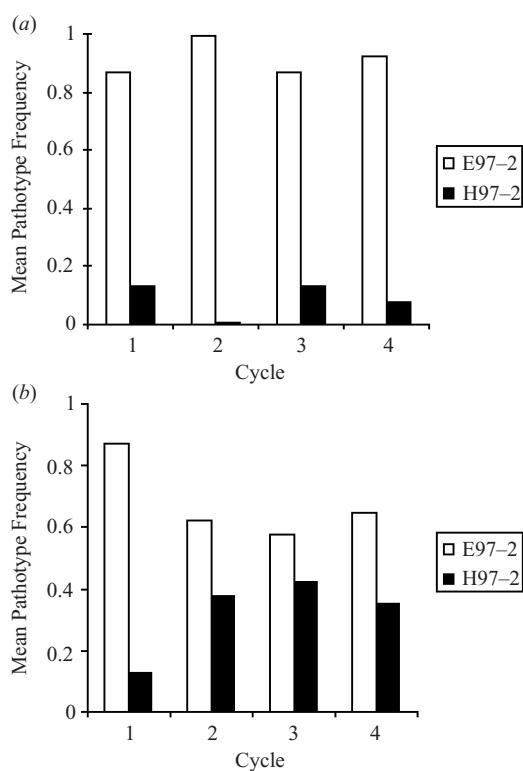
RAPD profiles were transformed to binary codes based on the presence or absence of amplified products of the same size at about 10 bp resolution generated from the fungal isolates by a single primer. The SIMQUAL program was used to estimate genetic distances based on Nei & Li (1979). Cluster analysis was performed on genetic distances using unweighted pair grouping by mathematical average algorithms (UPGMA) from Dice similarity values (Sneath & Sokal 1973). Based on the similarity coefficient, phenograms were then constructed using the SAHN program of the NTSYS-pc package (Rohlf 2000). The validity of the resulting clusters was confirmed using the COPH and MXCOMP programs in the NTSYS-pc software package.

## RESULTS

### Pathotype frequency

On average, isolate E97-2 showed five times as much sporulation as H97-2 *in vitro* and one to twice as much *in vivo*. Both isolates reached approximately 60% spore germination after 48 h incubation and there was no significant difference in spore germination between the two pathotypes. When inoculated singly, isolates E97-2 or H97-2 each resulted in about 90% frequency of isolation over the four cycles, while avirulent isolates accounted for 10% of the frequency on either host. When the two isolates were mixed and inoculated, undetermined pathotypes based on the three cultivars accounted for an average of 10% of the populations in each cycle, indicating the occurrence of new pathotypes. An additional 10% of the colonies on average were avirulent on either host. Among the recognized pathotypes, E97-2 comprised more than 80% in the mixed population after one cycle when the original two-isolate mixture was inoculated on and isolated from cv. 'CDC Earl', and this high frequency of E97-2 remained throughout all four cycles (Fig. 1A). Pathotype H97-2 was found at a frequency of <12% in all four cycles following the mixed inoculation on 'CDC Earl', indicating that this cultivar differentiated the two pathotypes by favouring E97-2 over H97-2. In the mixed population inoculated on and isolated from cv. 'Harrington', pathotype E97-2 comprised >80% of the isolates in the mixed population after the first cycle, and about 60% of the isolates for the three remaining cycles (Fig. 1B). Although H97-2 had a relatively low frequency in the first cycle, it then tended to increase and comprised about 40% of the isolates found in the next three cycles.

The differences in pathotype frequency were significant with E97-2 predominating over H97-2 in the mixed population (Table 1). There was a significant interaction between pathotype and host cultivar, resulting from the relatively high frequency of H97-2 on



**Fig. 1.** Frequency of pathotypes E97-2 and H97-2 of *Rhynchosporium secalis* co-inoculated over four cycles on cvs 'CDC Earl', susceptible to E97-2 (a) and 'Harrington', susceptible to both (b).

cv. 'Harrington' compared with its frequency on cv. 'CDC Earl' (Figs 1A and 1B). This suggests that H97-2 competed better on its original host cv. 'Harrington' than on cv. 'CDC Earl'. The hypothesis that the orthogonal components in the multivariate data were uncorrelated was not rejected based on the Sphericity test ( $P=0.36$ ). Consequently, a valid conclusion can be drawn that there was a significant interaction between cycle and pathotype (Table 2). This interaction probably resulted from the difference in pathotype frequency over the four cycles on cv. 'Harrington', with E97-2 decreasing somewhat, while H97-2 increased slightly over time (Fig. 1B).

### Molecular phenotype

Classification of the molecular phenotype for each of the individual isolates and their mixture was made using ten random primers that produced at least one polymorphic fragment per primer. An example of the RAPD patterns is shown in Fig. 2. With primer OPA20, a PCR product of 0.6 kb was present in all single and mixture isolates of H97-2 derived from cvs 'Harrington' and 'Manley', but absent in those derived from cv. 'CDC Earl'. With OPX7, a PCR product of 1.4 kb was present in H97-2, but absent in E97-2. A product of 2.6 kb was present in all mixtures derived from inoculations on cv. 'Harrington', but absent in the mixtures derived from inoculations on cv. 'CDC

**Table 1.** Repeated measures analysis of variance (tests of hypotheses for between subject effects) with arcsine transformation for the frequency of two pathotypes of *Rhynchosporium secalis* on barley cvs 'CDC Earl' and 'Harrington' co-inoculated for four cycles.

Source	DF	SS	MS	F value
block	3	6.1	2.0	0.0
cultivar	1	0.8	0.8	0.0
pathotype	1	38502.6	38502.6	46.2**
cultivar * pathotype	1	4275.3	4275.3	5.1*
error	9	7487.6	832.0	

\*, \*\* Significant at  $P=0.05$  and  $0.01$ , respectively.

**Table 2.** Univariate analysis of variance (tests of hypotheses for within-subject effects) with arcsine transformation for frequency of two pathotypes of *Rhynchosporium secalis* on barley cvs 'CDC Earl' and 'Harrington' co-inoculated for four cycles.

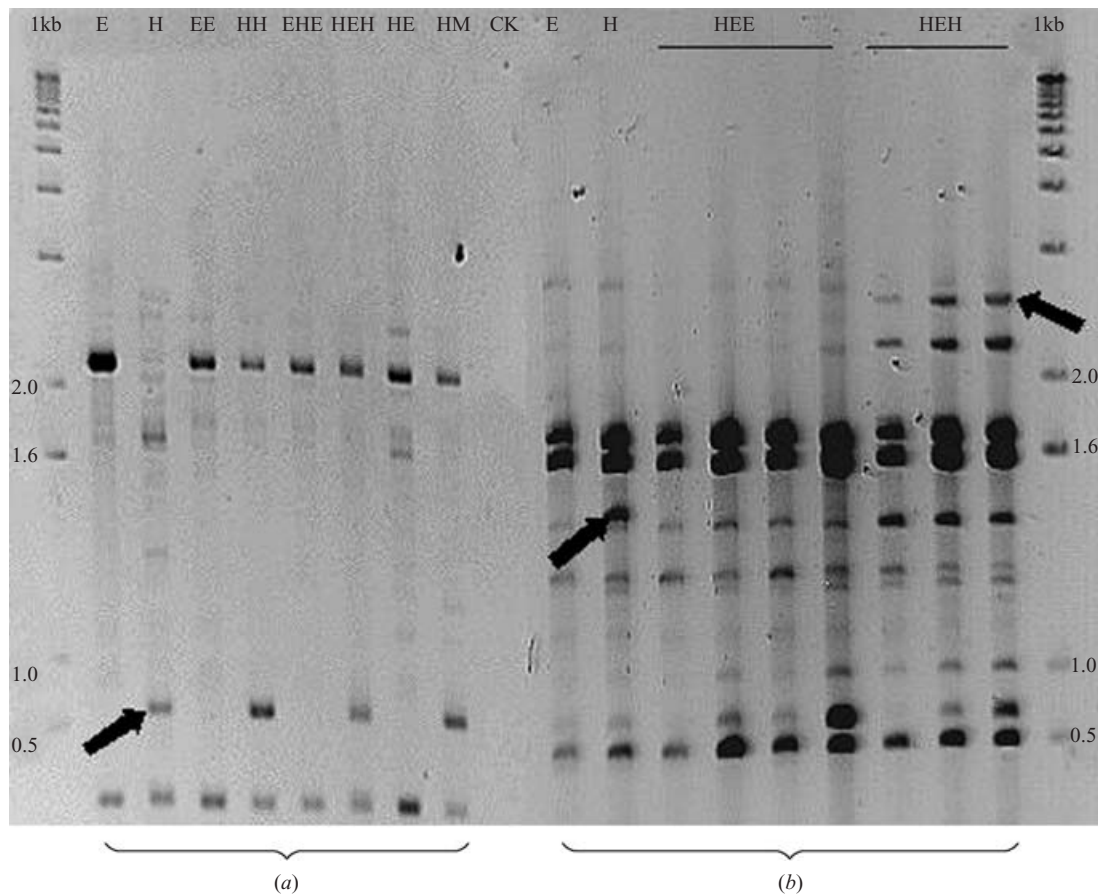
Source	DF	SS	MS	F value
cycle	3	1.2	0.4	0.0
cycle * block	9	1.6	0.2	0.0
cycle * cultivar	3	0.4	0.1	0.0
cycle * pathotype	3	1922.8	640.9	3.3*
cycle * cultivar * pathotype	3	1679.4	559.8	2.9
error	27	3287.1	195.8	

\* Significant at  $P=0.05$ .

Earl'. The number of polymorphic fragments produced with each of the ten primers varied from 1 to 12 per isolate and 94 loci, based on the presence or absence of amplification products ranging in size from 300 to 3000 bp, were used for cluster analysis. The molecular phenotypes derived from the two original isolates were grouped into two major clusters (Fig. 3). Except for HEH2, the upper cluster contains H97-2 and its derivatives that were grown on cv. 'Harrington'. The bottom cluster contains E97-2, its derivatives and the inoculation mixtures that were grown on cvs 'CDC Earl' or 'Harrington'. The majority of the mixed phenotypes, except for HEH2, were classified as being similar to E97-2, and this was in agreement with the pathotype frequency test where E97-2 dominated over H97-2 in the mixed populations. The classification of one colony, HEH2, into the molecular phenotype of H97-2 group indicated either that a change towards H97-2 occurred, but at only a low frequency or that such a classification occurred by chance. The same pairs of phenotypes, HEE1 and 3, EH1 and 2, EH3 and 4 were at the closest distance in similarity. However, misclassification occurred within the bottom group where HEH1 was separated from HEH4 and HEE2 was separated from HEE4.

## DISCUSSION

In this study, host genetic resistance or susceptibility was shown to result in a shift in the composition of *Rhynchosporium secalis* pathotypes. Cultivar 'CDC Earl' with specific host resistance strongly selected for pathotype E97-2, but against H97-2, resulting in the

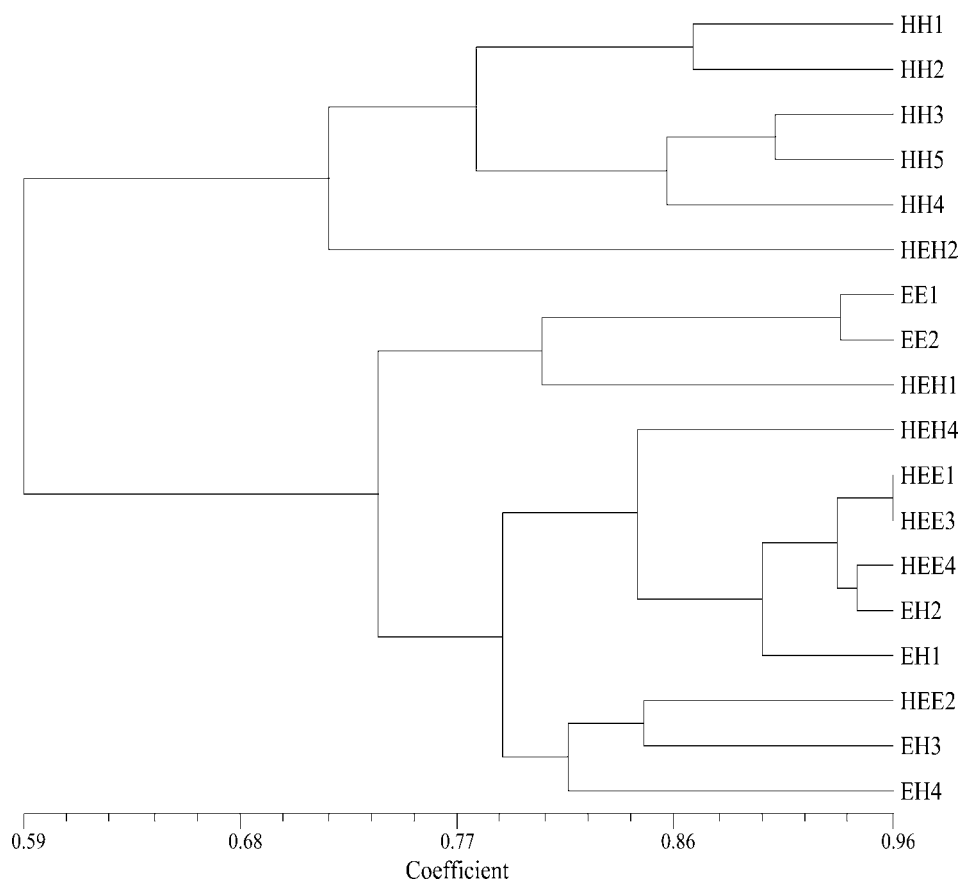


**Fig. 2.** RAPD analysis showing amplification patterns for E97-2 isolated from 'CDC Earl' and inoculated onto 'CDC Earl' (isolate E); H97-2 isolated from 'Harrington' and inoculated onto 'Harrington' (isolate H); E97-2 back-inoculated onto 'CDC Earl' twice (isolate EE); H97-2 inoculated onto 'Harrington' twice (isolate HH); E97-2 and H97-2 isolated from 'CDC Earl' and 'Harrington', respectively, and mixture inoculated on 'CDC Earl' (isolate EHE); H97-2 and E97-2 isolated from 'Harrington' and 'CDC Earl', respectively, and mixture inoculated on 'Harrington' (isolate HEH); H97-2 isolated from 'Harrington' and inoculated on 'Manley' (isolate HM); H97-2 isolated from 'Harrington' and inoculated on 'CDC Earl' (isolate HE); H97-2 and E97-2 isolated from 'Harrington' and 'CDC Earl', respectively, and mixture inoculated on 'CDC Earl' (isolate HEE); blank (CK). (a) amplification using OPA20; (b) amplification using OPX7. Sizes of main bands are indicated on both side lanes in kilobase pairs. Arrows indicate three different RAPD loci.

predominance of the former and a low level of the latter in the mixed population. Selection pressure exerted by 'CDC Earl' and other resistant cultivars may explain the occurrence of certain predominant pathotypes in barley fields of Alberta. Several resistant cultivars, including 'CDC Earl' bred in western Canada, are considered to have the same scald reaction based on genetic analyses and greenhouse inoculations (Penner *et al.* 1996). Furthermore, these cultivars have been classified into the same scald reaction group based on multiple location-season evaluations across Alberta (Xi *et al.* 2003). In a separate study based on samples from 1997 and 1998, E97-2 and its related pathotypes comprised only 21% of the total isolates in Alberta as opposed to 46% for H97-2 (Xi *et al.* 2002a). However, a more recent survey indicated that there was an increase in frequency of E97-2 and related pathotypes across Alberta and that they occurred in regions where they had not been detected previously (Xi *et al.* 2002b). The reproductive advantages of E97-2 and its current

status as one of the major pathotypes in Alberta indicate that this pathotype and possible future variants will continue to impact on barley production in this region. The relatively low frequency of E97-2 compared with H97-2 in previous field studies (Xi *et al.* 2000) may be explained by the fact that cv. 'Harrington' accounted for 50–60% of the total acreage seeded to barley in some regions (Edney & Tipples 1997). Thus, pathotype H97-2 will likely remain in the *R. secalis* population as long as the susceptible cv. 'Harrington' is grown over a large acreage in Alberta. Both cultivar and environment will continue to influence population shifts in the scald pathogen. Growing cultivars that have a broad genetic basis for resistance may minimize changes in virulence of the scald pathogen.

It has been suggested that races of an organism with the least number of unnecessary virulence genes are the most frequent in the population (Flor 1956). van der Plank (1968) supported this concept by proposing 'stabilizing selection' whereby a pathogen loses fitness



**Fig. 3.** Phenogram using UPGMA (unweighted pair group method, arithmetic mean) and RAPD procedures showing similarity coefficients in single or mixed pathotypes of *Rhynchosporium secalis* derived from cvs 'Harrington' and 'CDC Earl' and inoculated on cvs 'Harrington' and 'CDC Earl'. Five isolates (HH1-5) derived from H97-2 isolated from cv. 'Harrington' and inoculated on 'Harrington' twice; two isolates (EE1-2) derived from E97-2 isolated from cv. 'CDC Earl' and inoculated on 'CDC Earl' twice; four isolates (EH1-4) derived from E97-2 isolated from cv. 'CDC Earl' and inoculated on 'Harrington'; three isolates (HEH1, 2, 4) derived from H97-2 and E97-2 isolated from cv. 'Harrington' and 'CDC Earl', respectively, and mixture inoculated on 'Harrington'; four isolates (HEE1-4) derived from H97-2 and E97-2 isolated from cv. 'Harrington' and 'CDC Earl', respectively, and mixture inoculated on 'CDC Earl'.

when gaining virulence genes against plants having major genes for resistance. Thus, races possessing unnecessary virulence genes should decrease in frequency from reduced fitness compared with those without unnecessary virulence genes. In the present study, E97-2, the pathotype with unnecessary virulence genes against cv. 'Harrington' maintained a relatively high frequency over H97-2. This suggests that E97-2, a pathotype with unnecessary virulence genes against a susceptible cultivar, had greater parasitic fitness compared with H97-2, a pathotype without unnecessary genes for virulence. The presence of unnecessary virulence genes was demonstrated in the *R. secalis* populations of Idaho and Oregon (Goodwin *et al.* 1992), Norway (Salamati & Tronsmo 1997) and Denmark (Jorgensen & Smedegaard-Petersen 1995) and that these isolates were capable of overcoming resistance not used in Norway and Denmark. Likewise, Jackson & Webster (1976a) found that their results were not in agreement with the concept of a positive association between race simplicity and race fitness. These findings were in contrast with the theory of stabilizing selection proposed

by van der Plank (1968). However, in another study, Jackson & Webster (1976b) supported this concept by demonstrating that there was a shift in the pathogen population away from more complicated races towards simpler pathogenic ones. Zhang *et al.* (1987) demonstrated that aggressiveness of *R. secalis* races was negatively associated with racial complexity, while similar results were found by Ali (1975), Williams & Owen (1975). Xue & Hall (1991) also supported the theory by showing that less virulent races have high parasitic fitness.

This study showed that E97-2, a complex pathotype, can be a good competitor compared with H97-2, a simple pathotype. The competitive advantage of E97-2 may play a role in maintaining its proportion in the *R. secalis* population of Alberta regardless of whether it possesses unnecessary genes. Watson (1970) proposed a model operating in plant pathogens interacting with host plants where virulence is controlled by specific genes under a relatively simple genetic system; while growth rate, lesion size, sporulation, aggressiveness and characters related to pathogenicity are controlled by a

polygenic system. It would be informative to determine how the interaction between these two systems may play a role in influencing the composition of individual strains in a pathogen population.

In a previous study, Johnson, Penner & Tekauz (1996), found that isolate grouping based on virulence was not associated with RAPD polymorphic banding patterns among *R. secalis* isolates. They considered that the lack of correlation resulted from DNA sequence variation that is spread throughout the entire genome and is not confined to regions associated with virulence or avirulence. However, in the present study, the molecular phenotypes generally reflected the presence of the two virulent phenotypes. The use of *R. secalis* pathotypes differing widely in virulence, as in the current study, may facilitate the detection of RAPD polymorphisms, thereby enhancing the correlation between virulence and molecular phenotypes. The mixed inoculations resulted in different molecular phenotypes, indicating that changes occurred at the molecular level for the pathotypes studied. Based on isozyme analysis, somatic recombination (Burdon *et al.* 1994), and mutation and migration (Goodwin, Webster & Allard 1994) were considered to be contributing factors to the high degree of genetic variability in *R. secalis*, as was asexual recombination, possibly during a parasexual cycle (Newman & Owen 1985). In the present study, the new molecular phenotypes identified based on RAPD patterns suggested that there was mutation or somatic segregation between the two pathotypes under investigation.

The use of a single race that was found to prevail in the scald population has been suggested for screening scald resistance (Ceoloni 1980). However, the diversity in virulence of this pathogen suggests that breeding lines evaluated in a field nursery or in the greenhouse be screened against several isolates representing local pathotypes. The present study demonstrated that the effect of a mixture of individual isolates on scald reactions was partly masked by the presence of a predominant pathotype in the mixture. Tekauz (1991) also indicated that the overall effect of pathogen isolate mixtures is unknown. Thus, the overall effect of a mixture of isolates may need to be determined before isolates are mixed when screening for resistance.

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Corresponding Editor: H. T. Lumbsch