MOLECULAR GENETIC DIFFERENCES BETWEEN ISOLATES OF LEPTOSPHAERIA MACULANS

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INTRODUCTION

Leptosphaeria maculans (Desm.) Cas et de Not.[anamorph: Phoma lingam (Tode ex Fr.) Desm.] is the causal organism of blackleg disease of oilseed rape (Brassica napus L.) and other crucifers (Punithalingham and Holliday, 1972). Differences in the pathogenicity of isolates of L. maculans on rapeseed (Cunningham, 1927) led to a two strain classification system, highly (HV) and weakly virulent (WV). The two strains have notable differences in growth rate (Delwiche, 1980), pigment production (McGee and Petrie, 1978), allozymes (Hill et al., 1984), and phytotoxin production (Pedras et al., 1990). These data coupled with the failure in all attempted matings of the two strains (Delwiche, 1980; Bonman et al., 1981) has led to speculation that the two strains may actually be different species.

We used karyotyping by pulse field gel electrophoresis (PFGE) to clarify the relationship between highly and weakly virulent isolates of L. maculans.

MATERIALS AND METHODS

Isolates

Table 1 lists the geographic origin and source of the isolates used in these studies. The Canadian isolates were generously provided by Dr. G.A. Petrie and Mr. R.K. Gugel (Agriculture Canada Research Station, Saskatoon, Saskatchewan) and the two Western Australian isolates were kindly provided by Dr. S.R. Rimmer (Dept. of Plant Science, University of Manitoba, Winnipeg, Manitoba).

Fungal Culture and Pathogenicity Tests

The fungal isolates were cultured and tested for pathogenicity on the susceptible B. napus cultivar Westar as described in Gugel et al. (1990).

Protoplast preparation and PFGE

Spore suspensions were prepared as described (Gugel et al., 1990). The protoplast preparation method was modified from Specht et al.(1988).The modifications to the protoplast preparation procedure and the method for releasing intact chromosomes have been described elsewhere (Taylor et al., in press). The samples were loaded into a 0.6% (w/v) agarose gel and the wells were overlaid with agarose.

Saccharomyces cerevisiae chromosomes were used as molecular weight markers in the gels. The gel and running buffer were as described (Geneline Instruction Manual). The chromosomes were separated by transverse alternating field electrophoresis in the "Geneline System" from Beckman Instruments. The following conditions were used: 1) 40 V, 4 h, 20 min pulse; 2) 60 V, 186 h, 8 min pulse (Taylor et al., in press). The running buffer was maintained at 12°C

Phylogenetic Analysis Using Parsimony (PAUP).

The molecular sizes of the chromosomes of each isolate were calculated based upon linear migration of the S. cerevisiae chromosome standards. The data were computer analyzed using the PAUP, 2.4 program (Swofford and Berlocher, 1987). The data file input for the PAUP program had the following option settings: NOTU-22; NCHAR-71; User-tree-No; HYPANC-1; ADDSEQ-closest; HOLD-5; SWAP-alt; MULPARS-yes; OPT-n/a; ROOT-midpoint; Weights applied-no; OUTWIDTH-80; Missing data code-none; MAXTREE-50. All characters were unordered.

RESULTS

Electrophoretic Karyotpying

The electrophoretic separation of whole chromosomes from one HV and one WV isolate of L. maculans is shown in Fig. 1. The pattern of bands are dramatically different. The chromosome preparation from the HV isolate LEROY separated into 8 distinct bands; one band at a molecular size of $\sim$ 620 kb and all remaining bands at $>$ 1140 kb. The chromosome preparation of the WV isolate UNITY, however, separated into 12 distinct bands which migrated in a continuous array from $\sim$800 kb to 1900 kb. Bands which stained more intensely than others with ethidium bromide were present in both isolates and may indicate the presence of more than one chromosome of similar size. Chromosome preparations from 20 additional HV and WV isolates were examined to determine if the observed band migration differences were consistent. A diagrammatic representation of the band patterns found is shown in Fig. 2. The sizes and the resolvable number of chromosomes varied among isolates depending on their geographic origins. However, the chromosomes of all HV isolates separated into 6-8 bands in patterns similar to LEROY. Those of WV isolates separated into 12-14 bands in patterns similar to UNITY.

Phylogenetic Analysis using Maximum Parsimony

A consensus phylogenetic tree was generated by the Contree program (see M & M) and is presented in Fig. 3. There were only two HV and WV isolates which were closely associated on the tree, WA51 and WR5 with PEACE 3 and N. BATTLEFORD 2, respectively.

CONCLUSIONS

Despite some variation, an overall band pattern for highly virulent isolates and one for weakly virulent isolates is evident in PFGE. These patterns are definitely distinct from one another and indicate that the two strains are, in actuality, separate species. This conclusion is supported by the results of the phylogenetic analysis of the data. The implications of the association on the phylogenetic tree of two HV with WV isolates is unclear. The isolate WR5 from Ontario consistently produced lesions that were about half as

large as those produced by LEROY (Gugel et al., 1990). The lesions produced by the Western Australian isolate WA51 were also not consistent with those produced by LEROY (R.K. Gugel, unpublished).

Acknowledgements.

This work was partially funded by a grant from the NRC President's Fund. We thank Mr. R.K. Gugel and Dr. G.A. Petrie for determining the virulence of the fungal isolates. We also thank Dr. T.K. Blake from the Dept. of Plant and Soil Science, Montana State University for writing the input data file and running the PAUP program.

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Table 1. Source and geographic origin of isolates of Leptosphaeria maculans

ISOLATE	SOURCE	GEOGRAPHIC ORIGIN
HIGHLY VIRULENT:		
Fairview 1	Stubble	Fairview, Alberta
Fairview 2	"	" "
Leroy	Seed	Leroy, Sask.[a]
North Battleford 1	"	N. Battleford, Sask.
Saskatoon 7	Stubble	Saskatoon, Sask.
Saskatoon 8	"	Saskatoon, Sask.
Scott 1	"	Scott, Sask.
WA51	-	Western Australia
WA74-4	-	Western Australia
Wainwright 1	Seed	Wainwright, Alberta
WR5	Stubble	Ontario
WEAKLY VIRULENT:		
Peace 7	Stubble	Peace River, B.C.[b]
Peace 2	"	" " , "
Peace 3	"	" " , "
Peace 4	"	" " , "
Peace 5	"	" " , "
Lethbridge 1	"	Lethbridge, Alberta
North Battleford 2	Seed	N. Battleford, Sask.
North Battleford 3	"	N. Battleford, Sask.
Outlook 2	Stubble	Outlook, Sask.
Saskatoon 1	"	Saskatoon, Sask.
Unity	Seed	Unity, Sask.

a) Saskatchewan
b) British Columbia

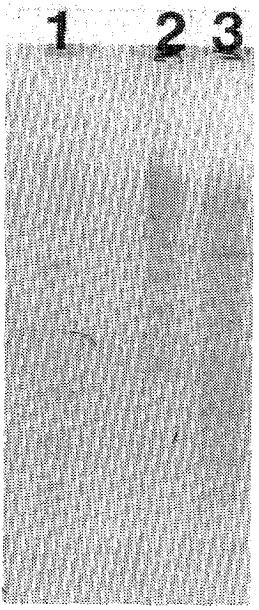

Fig. 1. PFGE separation of chromosomes from one HV and one WV isolate, respectively, of L. maculans. (1) S. cerevisiae standards; (2) LEROY; (3) UNITY

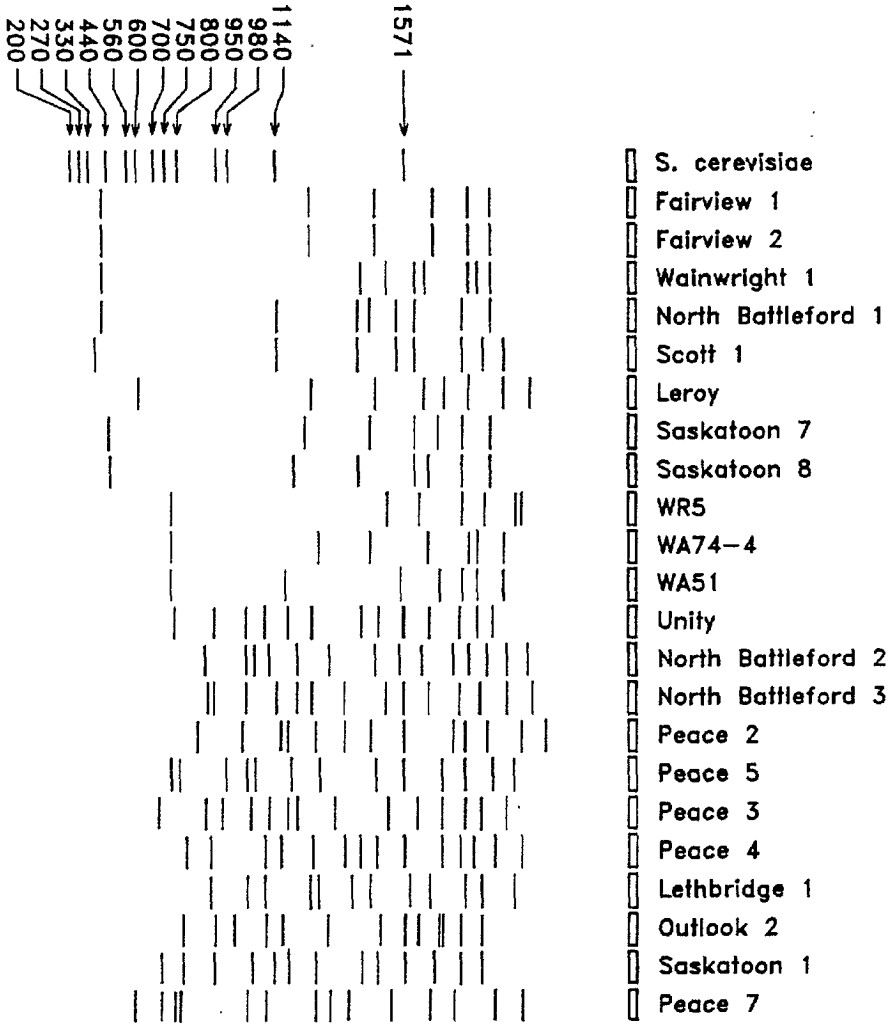

Fig. 2 Diagrammatic representation of the PFGE separation of chromosomes from 11 HV and 11 WV isolates of L. maculans.

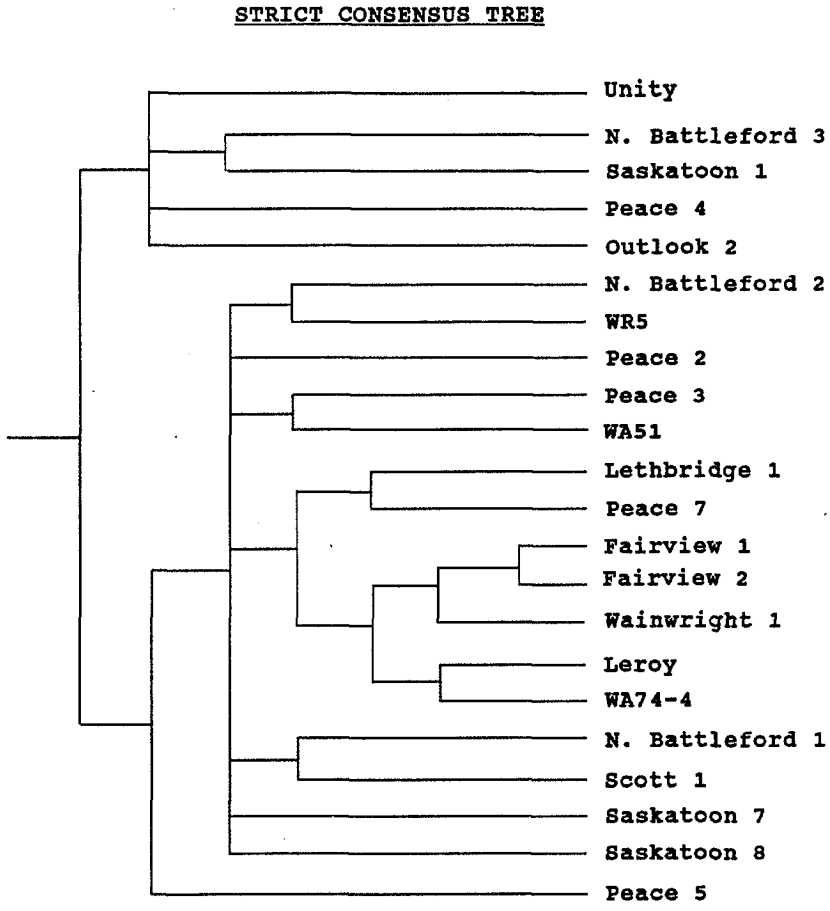

Fig. 3. Strict consensus phylogenetic tree of L. maculans isolates based on chromosome molecular sizes.