ISOENZYME STRUCTURE OF *CRONARTIUM RIBICOLA* IN EASTERN CANADA

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Summary

We conducted a genetic study of white pine blister rust fungus populations (Cronartium ribicola) using isoenzymes as genetic markers. Electrophoresis of 11 isoenzyme systems of 155 single-canker aeciospore samples collected from 21 white pine populations (*Pinus strobus*) in Québec and Newfoundland revealed that *C. ribicola* populations in eastern Canada are composed of only one zymodeme (multi-locus electrophoretic types). Within the zymodeme, all 21 loci studied were monomorphic (0.95 criterion), although variants were found at low frequencies (SYMBOL 163 \f "Greek Math Symbols"0.03) at two loci. Little variation is consistent with the recent introduction of this disease from Europe at the beginning of this century. The variable loci are found in populations located at the extreme west of the territory sampled. These could be indications that these populations may be at the limit of another zymodeme extending westward toward central Canada. Consequences of such reduced genetic variability on breeding programs aimed at selecting resistant white pine to the disease and the strategies for developing biocontrol methods are discussed.

Key words: White pine blister rust, *Cronartium ribicola*, isoenzymes.

Introduction

Knowledge of the genetic structure of a fungal pathogen population is important to understanding the potential for variability in virulence and other traits, and is crucial in determining disease control strategies. Some populations of crop pathogens have been characterized using isoenzyme analysis (Burdon et al. 1983, 1982). This technique has also been used to characterize wild populations of endemic forest pathogens such as *Peridermium harknessii* (Vogler et al. 1988; Tuskan and Walla, 1990) and *Armillaria* spp. (Rizzo and Harrington, 1993). Genetic structure of a recently introduced forest pathogen has not been studied previously and may lead to an understanding of the manner in which introduced pathogens invade new territory.

For this study, we examined the genetic structure of white pine blister rust fungus (WPBR), *Cronartium ribicola* J.C. Fischer ex Rabh., a widespread pathogen causing perennial cankers on stems and branches of 5 needle ("white") pines such as *Pinus strobus* L. in eastern Canada. WPBR was introduced to western North America in 1910, presumably from seedlings imported from France. It rapidly spread across the continent reaching the east coast by 1916 (Piché, 1917), decimating white pine populations with high mortality on young trees (Pomerleau 1932).

For decades, white pine planting programs were cancelled due to the high incidence of infection. By the late sixties, many researchers recommended new planting programs based on data indicating that in favourable zones, infection

rates were within acceptable limits (Lavallée, 1986). Considering the many hyperparasites of C. ribicola and parasites of Ribes spp., its alternate host and the complex life cycle of WPBR, a biological control program is part of a sensible strategy when combined with genetic improvement of white pine and selection of favourable planting sites.

Initiation of a biological control program necessitates the understanding of the genetic structure of populations of WPBR in eastern Canada. Isozymes are neutral biochemical markers that are useful to efficiently estimate the amount of genetic variability in fungi populations (Clark et al. 1989; Bonde et al. 1988; Leung and Williams 1986). In this study, we used isoenzymes to explore the genetic structure of *C. ribicola* collected from galls on P. strobus in Quebec and Newfoundland. Our goal was to characterize the genetic structure of WPBR throughout the eastern part of its range.

Material and Methods

Aeciospores were collected from galls on pines in the province of Quebec along the St. Lawrence river, from the Ottawa river valley to the Gaspé Peninsula. Spores were also collected in Newfoundland, from the western, central and eastern parts of the island (Figure 1 and 2). Newfoundland is the northern limit of white pine populations in eastern North America.

Aeciospores were collected by vacuum from beneath freshly-broken aecidia of single galls using Millipore filters. In the field, spores were stored under ice in sealed collecting vials. Spores were air-dried in vials for 24 hours in a dessicator at 4°C. Samples were weighed and stored at -20°C in screw-top polypropylene vials. To test viability, spores were dispersed by a blower onto 1.5% water agar in a Petri dish and germinated in the dark for 24 hours at 19°C. Germination was usually equal or higher than 95%.

A total of 155 spore samples were collected from 21 white pine stands (Table 1). Although samples were from single galls, they may not be genetically homogeneous since they may have originated from multiple sexual fertilization. Isozyme extraction was done using 25 mg of spores in 400 µL of extraction buffer (60 mM Tris, 20 % glycerol, pH 6.8) and ground by hand in a glass tissue grinder for 5 minutes under ice. The homogenate was pipetted into a microfuge tube and centrifuged at 15 000 G for 10 minutes. The supernatant then separated in aliquots of 25 µL and frozen until used. Aliquots, when thawed, were used only once.

Electrophoresis was performed on a Pharmacia PhastSystem® with native PhastGel Homogeneous 20 polyacrylamide gels. One µL of extract was loaded per well, migrated for 30 minutes and stained for the chosen enzyme system according to Cheliak and Pitel (1984).

Isoenzyme bands (phenotype) were scored from stained and fixed gels. Since formal genetic analysis of isozyme phenotypes was not possible in the absence of controlled crosses, homozygous and heterozygous genotypes were inferred whenever possible by comparing phenotypes with patterns expected of the same enzymes as described for fungi (Burdon et al. 1986, Bonde et al. 1988, Vogler et al. 1988), plants (Odrzykoski and Gottlieb, 1984) or animals (Harris and Hopkinson, 1978).

Results

Isoenzyme stains were obtained from 13 enzyme systems (Table 2). However 2 systems (fumarate hydratase and mannose phosphate isomerase) stained inconsistantly within and among isoenzymes runs and were not scored. Of the remaining 11 enzyme systems, well-resolved electromorphs for 20 putative loci in most aeciospores samples were obtained. Eighteen isoenzyme loci (6PGD1, GOT1, IDH1, IDH2, G-6-PD1, PGI1, PGM1, PGM2, AK1, AK2, ACO1, ACO2, MDH1, MDH-NADP1, MDH-NADP2, EST1, EST2, EST3) were monomorphic (0.95 criterion) for all samples. The simple banding patterns suggest that these eighteen isoenzymes are products of similar alleles and their respective loci are homozygous.

Two isoenzyme loci were polymorphic (MDH2, PGM 3) showing some variability in some aeciospore samples. The enzyme MDH, reported as being monomeric (Harris and Hopkinson, 1978; Goodman et al., 1980), had usually 1 band but exhibited 2 bands on three samples (FN 2, LD 1 and RB 6). These double bands were interpreted as a product of different alleles or allozymes and thus three samples were scored heterozygous. Another sample (SE 9) was homozygous but with a new allele. Similarly, PGM is reported being monomeric in *Puccinia* (Burdon et al., 1986) and exhibited only 1 band except for two samples (FN 2, P 8) which showed two bands. These were also interpreted as allozymes and scored as heterozygous.

Of the five samples showing some variation, only one sample (FN 2) from the western range of the sampled zone exhibited variation at more than one locus. The other four samples were variant for one locus only with no relation to their geographic distribution.

Discussion

Based on their electrophoretic profiles the samples tested belong to a single group. Such multi-locus phenotypes have been called zymodemes (Miles et al., 1977) a term used to characterize populations of identical multi-locus phenotypes. We therefore consider WPBR populations of eastern Canada to belong to the same zymodeme.

Tuskan and Walla, 1990) and Vogler (1988) research on western gall rust fungus *(Peridermiun harknessii)* demonstrated the presence of many zymodemes existing in western United States. We do not see such a situation in eastern Canada where only one zymodeme exists. Very limited variation at the western range of the sampled zone may suggest the beginning of another zymodeme, extending westward toward Ontario, the Prairies and the west coast.

Cronartium ribicola exhibits remarkably homogeneous isozyme genetic structure despite its wide range of alternative host species. This suggests *Ribes* and *P. strobus* impose little pressure for variation. As the North American host species did not coevolve with the fungus, P. strobus may not have developed resistance mechanisms that would have put selective pressure on the rust. Intensive selection programs in white pine have shown low resistance in the species. White pine species from Asia which have coevolved with WPBR offer some resistance (Hoff et al. 1980), but C. ribicola is a recently introduced organism to North America, and possibly may not yet have time to begin genetic differentiation. Genetic variation may occur but went undetected using isoenzyme

analysis. A population genetic study of *Septoria tritici* Roberge in Desmaz. (McDonald and Martinez, 1990) demonstrated the difference in average gene diversity between isoenzyme analysis (0.37) and RFLP analysis (0.45). Random Amplified Polymorphic DNA (RAPD) fragments have a potential to be even more sensitive and may show variation undetected with isozyme analysis. Limited isozyme variation may indicate lower variation in virulence genes which are not neutral like isozymes are thought to be, but strongly selected for. Variation in virulence genes may exist in C. ribicola but probably not to the extent of other fungal pathogens like *Septoria* (McDonald and Martinez, 1990).

The implications of genetic homogeneity of WPBR populations in eastern Canada for virulence are profound. The absence of variability suggests a limited number of virulence types. The enormous territory occupied by one zymodeme also limits the possibility of invasion by new genotypes. An invading zymodeme would not only be forced to colonize an occupied niche, but could also constantly see its virulence genes diluted by the enormous homogeneous gene pool surrounding it.

Genetic homogeneity of WPBR favours a biological control program and genetic breeding of white pine for resistance. Provided that pines bred for resistance are capable of hosting aeciospore producing cankers, the rust will be able to accomplish its life cycle. Thereby avoiding selection for new virulent strains. Moreover, given the fungus and its alternate host have many parasites and competitors, limited variation suggests a biocontrol program could succeed. The fungus may not have genetic variation to adapt to parasites or competitors. Conversely, the parasites and competitors will probably have a large genetic variability at their disposition due to their endemic presence on the continent for a long time.

A successful blister rust biocontrol program could facilitate the reintroduction of white pines to its previous range, reestablishment of plantations and renewed importance in our forest. In areas such as Newfoundland, where white pine has virtually vanished from its former range, reintroduction of this prolific species, unlimited by WPBR, could successfully increase biodiversity and reforestation of poor and marginal sites where white pine outcompetes every other species.

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Fig. 1. Geographic source of *C. ribicola* aeciospores samples collected from Newfoundland.

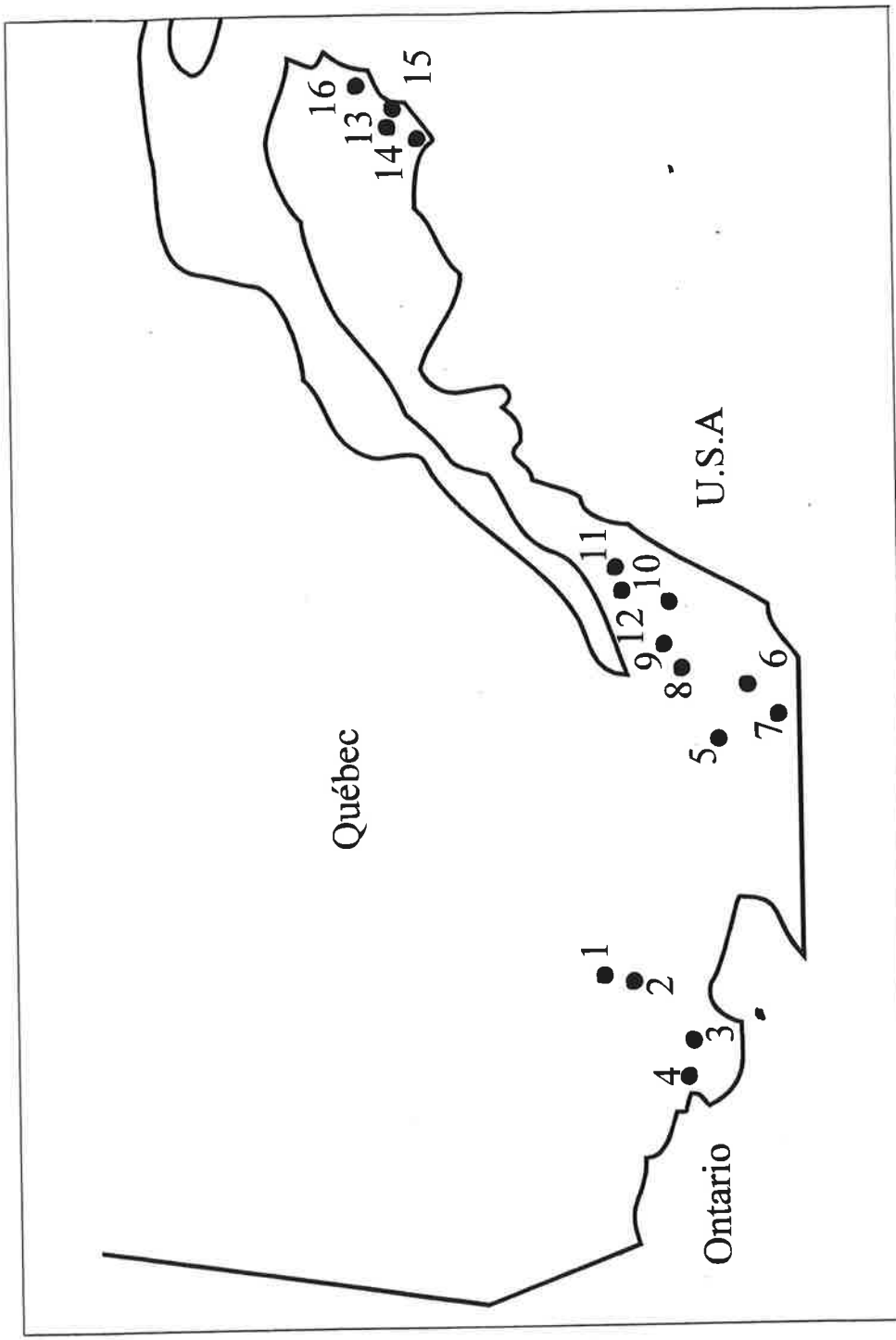

Fig. 2. Geographic source of *C. ribicola* aeciospores samples collected from Québec.

Table 1. Origin of *Cronartium ribicola* aeciospore samples collected in Quebec (Qc) and Newfoundland (NF).

Map number	Sample code name	Location
1	FN 1-10	Ferme-Neuve, Qc
2	LDI 1-8	Lac-des-Iles, Qc
3	BT 1	Belle-Terre, Qc
4	BN 1-3	Béarne, Qc
5	DS 3-11	Durham-Sud, Qc
6	SBL 4-8	Saint-Benoit-Labres
7	LB 3-8	Lac Brôme, Qc
8	P 3, 4, 7-10	Plessisville, Qc
9	SAR 2-10	Sainte-Anne-de-la-Rochelle, Qc
10	LS 1, 2	Lac Sergent, Qc
11	SM 3, 6-10	Sainte-Magloire, Qc
12	SPE 3, 5-7, 10	St-Perpetue
13	SE 6-11	St-Elzéar, Qc
14	BO 3-5	Bonaventure, Qc
15	RB 3-10	Riviere Baudet, Qc
16	LBA 3-10	Lac Baillargeon, Qc
17	L 1-15	Leewaseechieech, NF
18	LGL 1-8	Little Grand Lake, NF
19	H 1-9	Howley, NF
20	SHE 1-14	Sheffield Lake, NF
21	TN 1-13	Terra-Nova Village, NF

Table 2. Isoenzyme systems tested on *Cronartium ribicola* aeciospores samples.

Enzyme	Abbrev.	E.C. No.	Putative loci Obs.	Scored
6-phosphogluconate dehydrogenase	6-PGD	1.1.1.44	1	1
Glutamate-oxaloacetate transaminase	GOT	2.6.1.1	1	1
Isocitrate dehydrogenase	IDH	1.1.1.42	3	2
Malate dehydrogenase	MDH	1.1.1.37	3	2
Glucose-6-phosphate dehydrogenase	G6PD	1.1.1.49	2	1
Phosphoglucose isomerase	PGI	5.3.1.9	2	1
Phosphoglucomutase	PGM	2.7.5.1	3	2
Adenylate kinase	AK	2.7.4.3	2	2
Aconitase	ACO	4.2.1.3	2	2
Malate dehydrogenase-NADP	MDH-NADP	1.1.1.37	2	2
Esterases	EST	3.1.1.1	7	3
Total:			28	20
Enzyme systems that stained weakly				
Fumarase	FUM	4.2.1.2		
Mannose phosphate isomerase	MPI	5.3.1.8		