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Comparison of *Puccinia* spp. from *Carduus* Thistles Using Isozyme Analysis

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Abstract

Horizontal starch gel electrophoresis was used to test for the presence of 17 enzymes in germinated urediniospores of *Puccinia cardui-pycnocephali* from the host *Carduus pycnocephalus*, and *P. carduorum* from the hosts *C. acanthoides*, *C. tenuiflorus*, and *C. thoermeri*, respectively. An isolate of *P. carthami*, which is morphologically similar to the *Carduus* rust fungi, was included for comparison. Mean value for Rogers' Coefficient of Similarity (CS) test for isozyme patterns was 1.00 (= 100% correlation) for nine isolates from *C. thoermeri* collected in five countries in Eurasia. Similarly, CS for nine isolates from *C. pycnocephalus* from three countries was 1.00. Comparison of isolates from each *Carduus* species resulted in CS values of at least 0.70, indicating they are closely related. Comparison of isolates from the *Carduus* species with *P. carthami* resulted in an average CS value of 0.30. Rapid and positive identification of *Carduus* rust fungi without host plant information seems possible using this approach.

Introduction

Seven species of *Carduus* have been introduced into North America and most, if not all, are considered important as weeds (McCarty 1982). Among their natural enemies are rust fungi. All seven are susceptible to *Puccinia carduorum* Jacky, one is susceptible to *P. galatica* Syd., two to *P. hadacii* Urban, and three to *P. cardui-pycnocephali* Syd. (Watson and Brunetti, 1984). All of these rust species are members of the *P. centaureae - P. laschii* lineage (Savile 1970*a*), characterized by "broadly ellipsoid to spherical urediniospores with (2-)3(-4) generally equatorial germ pores". Other taxonomists do not agree with Savile on naming these rust fungi. Gaumann (1959) distinguishes between *P. carduorum* and *P. cardui-pycnocephali* and feels that *P. cardui-pycnocephali* and *P. galatica* are synonymous. Panditou (1969) feels that *P. galatica* and *P. cardui-pycnocephali* are synonyms of *P. carduorum*. Savile (1970*a*) recognizes *P. carduorum*, *P. galatica*, and *P. hadacii* as distinct, but he feels *P. cardui-pycnocephali* may be a variety of *P. carduorum*. Although much of the confusion results from difficulty in properly identifying the host (Savile 1970*a,b*, Watson and Brunetti 1984), it is evident that at least three of these species are very closely related.

Several other rust fungi in the *P. centaureae - P. laschii* lineage attack *Carthamus tinctorius* L. and native *Cirsium* species (both Asteraceae) in North America (Savile 1970*b*). These fungi as a group are difficult to distinguish on the basis of morphological features. A means for rapid and positive identification in the absence of host plant information would be very useful, especially considering the *Puccinia* species currently under evaluation for biological control of introduced *Carduus* thistles. One technique which has this potential involves the analysis of isozyme phenotype patterns.

Frequently, enzymes that act as catalysts for the same reaction are coded by more than one allele or genetic locus. As a result, gene products with similar enzymatic properties may possess slightly different amino acid sequences and therefore differ in electrophoretic mobility. Banding patterns that result from substrate-specific strains often are predictable for a given organism or species, because they are dependent upon the genetic or nuclear condition of the organism (Micales *et al.* 1986). Isozyme analysis is an inexpensive and versatile technique that relies on the direct manifestation of gene products. It is often more

reliable as a taxonomic or genetic tool than the use of morphological or physiological parameters, because it is less dependent upon environmental conditions.

The objectives of this study were to characterize isozyme banding patterns of rust fungi from four species of *Carduus* and to test the capability of this procedure for distinguishing these isolates from each other and from *P. carthami* Cda., an organism which is morphologically very similar to the *Carduus* rust fungi. Isolates from *C. acanthoides* L., *C. tenuiflorus* L., and *C. thoermeri* Weinm. were classified as *P. carduorum*; isolates from *C. pycnocephalus* L. were classified as *P. cardui-pycnocephali*. Thus, we were able to evaluate isozyme analysis by comparing isolates of related rust fungi from *Carduus* and one from *C. tinctorius* that is morphologically indistinguishable.

Materials and Methods

Comparisons were made among isolates of *P. carduorum* from six locations in Eurasia and the United States (U.S.), *P. cardui-pycnocephali* from three locations in Eurasia, and *P. carthami* from the U.S. The origin of isolates is detailed in Table 1.

Each isolate was increased in a containment greenhouse facility on the host species from which it was isolated. Isolates were increased in separate greenhouses or cubicles inside the facility to minimize the possibility of cross-contamination. Urediniospores were harvested with a cyclone spore collector, which utilizes a vacuum to remove ripe spores from infected plants. Urediniospores were prepared for isozyme analysis by floating them on distilled water for 6 hr at 20°C in a moisture chamber to induce germination. Germinated spores were separated from the water by suction filtration. The mat was divided into portions representing approximately 50 mg of ungerminated urediniospores, and each portion was placed in a 2.0-ml cryovial and frozen in a liquid nitrogen freezer. Samples were stored in liquid nitrogen until representatives of each isolate were ready for analysis.

The germinated urediniospores were crushed in the cryovials while still frozen using a 6 mm glass rod pre-cooled in liquid nitrogen. Two hundred fifty µl of 0.05 M TRIS/HCl buffer (pH 7.6) was added to each sample, and maceration continued until the sample was thawed. Each sample was transferred by Pasteur pipette to a 12 x 75 mm disposable glass test tube and held in an ice bath until centrifuged. Centrifugation was carried out at 1000 X G for 5 min. The supernatant of each sample was drawn into the required number of 4 X 12 mm filter paper wicks (#470, Schliecher and Schuelle, Keene, NH) and applied to the gels. Horizontal starch gel electrophoresis was performed as described by Micales *et al.* (1986).

Thirty-five enzymes in four buffers were screened with seven of the isolates in preliminary studies, and 17 enzyme-buffer combinations were found satisfactory for comparison of these isolates. Enzymes selected were [name (abbreviation, E.C. #)]: acid phosphatase (ACP, 3.1.3.2), aspartate aminotransferase (AAT, 2.6.1.1), diaphorase (DIA, 1.6.4.3), glucokinase (GK, 2.7.1.2), glucose-6-phosphate dehydrogenase (G6PDH, 1.1.1.49), glucose phosphate isomerase (GPI, 5.3.1.9), glutamate dehydrogenase (GDH, 1.4.1.3), glutathione reductase (GR, 1.6.4.2), glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 1.2.1.12), lactate dehydrogenase (LDH, 1.1.1.27), malate dehydrogenase (MDH, 1.1.1.37), malic enzyme (ME, 1.1.1.40), mannose phosphate isomerase (MPI, 5.3.1.8), peptidase (PEP-LA, 3.4.13), peptidase (PEP-LLL, 3.4.11), peptidase (PEP-PAP, 3.4.13), phosphogluconate dehydrogenase (PGD, 1.1.1.44), and purine nucleoside phosphorylase (NP, 2.4.2.1).

Three buffer systems were used during electrophoresis. The first, a continuous system described by Clayton and Tretiak (1972), was used to separate GK, G6PDH, GPI, and PGD at 200 v for 3 hr. The second, a discontinuous system described by Ridgeway *et al.* (1970), was used for separating AAT, GDH, GR, GAPDH, MPI, and PEP-LLL at 250 v for 3 hr, and the third was a discontinuous system modified by May (1980) to separate the remainder of the enzymes at 275 v for 3 hr.

Frequency of each allele was used to determine Rogers' Coefficient of Similarity (Rogers 1972), calculated by the FORTRAN program "Allozyme" (Strauss, personal communication).

Table 1. *Carduus* rust isolates compared by isozyme analysis.

Pathogen (*Puccinia* sp.)	Host	FDWSRU #	Collector	Country of Origin
cardui-pycnocephali Syd.	*Carduus pycnocephalus* L.	84-06	Bruckart	Italy
		84-07	Clement	Greece
		84-23	Rosenthal	Turkey
		84-58	Rosenthal	Turkey
		84-83	Bruckart	Greece
		85-18	Politis	Greece
		85-80	Politis	Italy
		85-128	Politis	Italy
		85-160	Politis	Greece
carduorum Jacky	*C. acanthoides* L.	RSL-108	Emge	Romania
	C. tenuiflorus L.	84-3	Supkoff	USA
	C. thoermeri Weinmann.	III	Emge	Turkey
		IV	Emge	Bulgaria
		V	Emge	Bulgaria
		VI	Emge	Bulgaria
		VII	Emge	Romania
		84-32	Bruckart	Italy
		84-40	Bruckart	Greece
		84-87	Bruckart	Greece
		84-88	Bruckart	Italy
carthami Cda.	*Carthamus tinctorius* L.	-	Klisiewicz	USA

Results and Discussion

Isolates from each of the *Carduus* species were found to be closely related, but they could be distinguished on the basis of isozyme phenotype patterns (Table 2). Coefficient of similarity (CS) was 1.00 (all alleles were the same) for all nine rust fungus isolates from *C. pycnocephalus* (Table 1), and CS also was 1.00 for the nine rust fungus isolates from *C. thoermeri* (Table 1). Reciprocal comparisons of isolates from each *Carduus* species also revealed a high degree of similarity, since CS value ranged from 0.72 to 0.95. Enzymes which enabled separation of the *Carduus* rust fungi were AAT, GK, GR, and MPI.

Table 2. Values for Rogers' Coefficient of Similarity between isolates of rust fungi compared using isozyme analysis.

Source of Isolate	*Carduus*				*Carthamus*
	aca.	*pyc.*	*ten.*	*tho.*	*tin.*
Carduus acanthoides L. (*aca.*)	1.00	0.72	0.75	0.72	0.32
C. pycnocephalus L. (*pyc.*)		1.00	0.90	0.89	0.32
C. tenuiflorus L. (*ten.*)			1.00	0.95	0.21
C. thoermeri (*tho.*)				1.00	0.32
Carthamus tinctorius L. (*tin.*)					1.00

Isozyme banding patterns were much different for *P. carthami*. CS values in comparisons of *P. carthami* and the *Carduus* rust fungi ranged from 0.21 to 0.32 supporting reports that *P. carthami* is a species distinct from those attacking *Carduus* thistles.

These results suggest that electrophoretic separation of enzymes from germinated urediniospores may be a quick and precise way to identify several rust fungi in the *P. centaureae - P. laschii* lineage. Presently, it appears possible to distinguish among isolates of rusts from four *Carduus* species, and it seems likely that isozyme analysis could be used for positive identification of isolates from *Cirsium* species in North America.

Results from this study also support earlier reports that *P. cardui-pycnocephali* may be synonymous with *P. carduorum* (Panditou 1969, Savile 1970*a*, Urban 1965). This study serves as a foundation for future comparisons between *P. galatica* and *P. hadacii*, which have uncertain affiliation with *P. carduorum*, and between the *Cirsium* rust fungi of North America in the *P. centaureae - P. laschii* lineage (Savile 1970*a*). The ability to identify pathogens of *Cirsium* and *Carduus* species in the absence of host plant information is needed to accurately determine if introduced *Puccinia* species are attacking native North American *Cirsium* species. Isozyme evaluation has potential applications in taxonomy and field evaluations, particularly with regard to monitoring spread of introduced pathogens for weed control.

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