Phenotypic Comparison of *Puccinia carduorum* from *Carduus thoermeri*, *C. tenuiflorus*, and *C. pycnocephalus*

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ABSTRACT

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Host range, urediniospore morphology, and isozyme banding patterns of field isolates of rust fungi from *Carduus pycnocephalus*, *C. tenuiflorus*, and *C. thoermeri*, collected in different geographic locations, were compared. Isolates from the three *Carduus* species are *Puccinia carduorum*, according to the isozyme data, supporting the view that *P. carduorum* is preferred to *P. cardui-pycnocephali* for isolates from *C. pycnocephalus*. Even though Rogers' Coefficient of Similarity supports the fact that the isolates are the same species, the isolates could be distinguished by isozyme banding patterns for aspartate aminotransferase and glucokinase. Banding patterns for glucose phosphate isomerase, malate dehydrogenase, and mannose phosphate isomerase also enabled distinction between the *Carduus* rusts and *Puccinia carthami* from *Carthamus tinctorius* (safflower). Differences in host preference also were noted for isolates from the three *Carduus* species. Isolates from *C. tenuiflorus* and *C. thoermeri* caused infection most on the species from which they originated, but isolates from *C. pycnocephalus* were more virulent on *C. tenuiflorus*. Urediniospores from *C. pycnocephalus* and *C. tenuiflorus* were significantly ($P = 0.05$) larger in volume (16 and 12%, respectively) than those from *C. thoermeri*. Also, the first two species had at least 10% more of the urediniospore surface covered with echinulations than did *C. thoermeri*.

Additional keywords: biological weed control.

Of seven species of *Carduus* that have been introduced into North America (19), *C. thoermeri* Weinm. (= *C. nutans* L., musk thistle) is the most widely distributed in the United States. It is a large-flowered species particularly important in Appalachia and the Midwest (8), where it displaces valuable forage and hay plants. *Carduus pycnocephalus* L. (Italian thistle) and *C. tenuiflorus* Curtis (slenderflower thistle) are closely related, slender-flowered species found mostly in California (8).

All three species are considered good candidates for biological control. *C. thoermeri* and *C. pycnocephalus* have been specifically chosen by the USDA-ARS as targets (35), and rust fungi in the genus *Puccinia* have been included among the candidates for biological control (1–3). Although differences between the rusts of *Carduus* have been reported in earlier studies on host range (10,24) and morphology (28,29), classification of these fungi remains unclear (36). The *Carduus* rusts have been classified either as a single species, *Puccinia carduorum* Jacky (22,28,29), or as one of three species (9,10,12–14,31,32,34).

The rusts of *Carduus* are morphologically similar and are included by Savile in the *Puccinia centaureae–P. laschii* lineage (28). This lineage includes the brachycyclic rusts on members of the tribe Cardueae in the Asteraceae. Several of these rust fungi occur in North America, including *Puccinia carthami* Corda, the cause of rust in *Carthamus tinctorius* L. (safflower). These species are very similar morphologically to *P. carduorum* (29). Despite the similarity of *P. carduorum* to other rusts, physiological specialization has also been described by Gaumann (10).

One objective of this study was to determine the relationship of rusts from *C. pycnocephalus* to those from other *Carduus* species. We also looked for ways to positively distinguish the rusts of *Carduus* from morphologically similar rusts that occur on related composites in North America. Distinguishing among the rust fungi of *Carduus* is the first step. In this study, *Puccinia* isolates from three *Carduus* species and safflower are compared by host range, urediniospore morphology, and isozyme banding patterns.

MATERIALS AND METHODS

Collection and maintenance of isolates. Ten field acquisitions (isolates) of *P. carduorum* from *C. pycnocephalus* and 10 from *C. thoermeri* were obtained from Italy, Greece, Turkey, and the Balkan Peninsula. Isolates from each of these hosts represented acquisitions from different locations in our collections. These isolates were compared with a California isolate of *P. carduorum* from *C. tenuiflorus*, a host very closely related to *C. pycnocephalus*. Also, a California isolate of *P. carthami* from safflower was included in certain studies as a representative of other similar rust fungi in the complex described by Savile (28).

Acquisitions were made between 1976 and 1985, and isolates were classified by the host plant from which the rust was collected. Host plant names provided by collectors were used for classification, since specimens usually did not include appropriate plant material for verification of host species (Table 1). Collectors who provided isolates are experienced with each of the plant species.

All 22 isolates used in this study were shipped to our containment greenhouse as pustules in dried leaf material. Each was preserved in a liquid nitrogen freezer upon receipt. Isolates were increased by heat-shocking frozen samples at 40 C for 5 min immediately upon removal from the freezer, washing inoculum from the leaf sample with freon, and inoculating plants of the same host species with the freon suspension by using an atomizer. Inoculated plants were given dew for 16 hr at 20 C. Pustules that developed in the greenhouse 2–3 wk later were harvested and stored in a liquid nitrogen freezer until sufficient quantities were collected for additional tests. Increases of inoculum and all of the comparisons were made in a containment greenhouse

(20) following protocols for evaluation of exotic plant pathogens (3,16).

Plants were inoculated for comparative studies as described, except that 0.1 mg of urediniospores was applied per plant as plants rotated inside a turntable settling tower. Germination of urediniospores was greater than 60% in each application. Data for disease severity and infection type were collected 3 wk after each inoculation. Details for disease severity (infection amount or IA) and infection type (IT) rating schemes have been published (1 and 24, respectively). Disease severity values range from 0 (no pustules) to 4 (more than 10 pustules on each of the three most infected leaves); values for infection type also range from 0 (no macroscopic symptoms) to 4 (very large pustules).

Data for infection type and disease severity were evaluated statistically by multivariate analysis using the MANOVA option in the general linear models procedure of the SAS statistical package (27). Least square means also were calculated during the analyses and compared using Fisher's LSD.

Reciprocal inoculations. Sets of plants that included one U.S. collection each of *Carduus acanthoides* L., *C. macrocephalus* Desf., *C. nutans* (after Kazmi [15]), *C. pycnocephalus* (collection number 27, most susceptible to all acquisitions from *C. pycnocephalus*), *C. tenuiflorus*, *C. thoermeri*, and *Cynara scolymus* L. (artichoke) were inoculated with the isolates from *C. pycnocephalus*, *C. tenuiflorus*, and *C. thoermeri*. Plants were inoculated 4–6 wk after planting, and five plants of each species were included in each set. Artichoke was included, because it was found to be susceptible to isolates of *P. carduorum* from *C. thoermeri* and *C. tenuiflorus* in earlier studies (4,24). We compared the susceptibility of artichokes to several isolates of the *Carduus* rusts under standardized conditions.

The relative susceptiblity of *C. pycnocephalus* from California was also determined in a separate study by inoculating seven collections of this species with four isolates of rust collected from *C. pycnocephalus* in Greece, Italy, and Turkey.

Isozyme analysis. Isolates selected for isozyme analysis were increased and stored as described. Urediniospores were germinated by floating them on distilled water for 6 hr at 20 C in a moist chamber. Germinated spores were collected by suction filtration and divided into portions, each approximately 50 mg, which were placed into 2-ml cryovials and frozen in a liquid nitrogen freezer until the time for analysis.

Before electrophoresis, germinated urediniospores were crushed in the cryovials, while still frozen, with a 6-mm glass rod precooled in liquid nitrogen. Two-hundred fifty microliters of 0.05 M Tris/HCl buffer (pH 7.6) were added to each sample and macerated until the sample was thawed. Samples were transferred to 12- × 75-mm disposable glass test tubes set in an ice bath before centrifugation at 1,000 *g* for 5 min. Supernatant from each sample was drawn into the required number of 4- × 12-mm filter paper wicks (#470, Schliecher and Schuell, Keene, NH) and applied to the gels. Horizontal starch gel electrophoresis was performed as described by Micales et al (21).

Thirty-five enzymes in four buffers were screened with seven of the isolates in preliminary studies, and 15 enzyme-buffer combinations were found satisfactory for comparison of these isolates. Enzymes selected were (name, abbreviation, EC): acid phosphatase (ACP, 3.1.3.2), aspartate aminotransferase (AAT, 2.6.1.1), diaphorase (DIA, 1.6.2.2), glucokinase (GK, 2.7.1.2), glucose-6-phosphate dehydrogenase (G6PDH, 1.1.1.49), glucosephosphate isomerase (GPI, 5.3.1.9), glutamate dehydrogenase (GDH, 1.1.1.47), 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 (5), was used to resolve bands of GK, G6PDH, GPI, and PGD at 200 V for 3 hr. The second, a discontinuous system, described by Ridgeway et al (25), was used to resolve AAT, GDH, GR, GAPDH, MPI, and PEP-LLL at 250 V for 3 hr. The third was a discontinuous system modified by May (17) to resolve the remainder of the enzymes at 275 V for 3 hr. Frequency of each putative allele was used to determine Rogers' Coefficient of Similarity (CS) (26), calculated by the FORTRAN program "Allozyme" (R. Strauss, *personal communication*).

The genetic nomenclature of May et al (18) was used in the presentation of results. Abbreviations of enzymes are capitalized (e.g., GPI) and putative alleles use the same abbreviation as the enzyme, but only the first letter is capitalized (e.g., Gpi-100).

TABLE 1. Isolates of *Puccinia carduorum* from *Carduus pycnocephalus*, *C. tenuiflorus*, *C. thoermeri*, and of *P. carthami* from *Carthamus tinctorius* (safflower) compared by host range, microscopy, and isozyme analysis

Isolate species	Host	Isolate no.	Collector	Origin	Study[a]
P. carduorum[b]	*C. pycnocephalus*	84-06	Bruckart	Italy	H, I, M
		84-07	Clement	Greece	H, I, M
		84-23	Rosenthal	Turkey	H, I, M, S
		84-58	Rosenthal	Turkey	H, I
		84-83	Bruckart	Greece	H, I, M
		85-18	Politis	Greece	H, I, M
		85-80	Politis	Italy	H, I, M, S
		85-128	Politis	Italy	H, I
		85-160	Politis	Greece	H, I, M, S
		85-171	Politis	Greece	M
P. carduorum	*C. tenuiflorus*	84-03	Supkoff	USA-CA	H, I, M, S
P. carduorum	*C. thoermeri*	78-02	Emge	Turkey	H
		78-03	Emge	Turkey	H, I, M, S
		78-04	Emge	Bulgaria	H, M
		78-05	Emge	Bulgaria	H, I, M
		78-08	Emge	Bulgaria	H, I, M
		78-09	Emge	Romania	H, I, M, S
		84-32	Bruckart	Italy	H, I, M
		84-40	Bruckart	Greece	H, I, M
		84-87	Bruckart	Greece	H, I, M, S
		84-88	Bruckart	Italy	H
P. carthami	*C. tinctorius*	SAF-1	Klisiewicz	USA-CA	H, I, M

[a] Codes for each study used on an isolate are: H = host range, I = isozyme analysis, M = morphology (urediniospore volume), S = scanning electron microscopy.

[b] *P. carduorum* from *C. pycnocephalus* has been classified also as *P. cardui-pycnocephali.*

The number following the allele designation refers to the relative mobility of the enzyme with a value of 100 being assigned to the most common allele for the enzyme. Enzymes that catalyze the same reaction, but that migrate not as far or migrate farther, are assigned proportionately smaller or larger numbers, respectively, to represent their relative mobility. For example, Gpi-100 is the most common allele coding for glucose phosphate isomerase, whereas Gpi-125 designates an allele coding for a variant of GPI that migrated 25% farther than the most common form.

Morphological determinations. Urediniospores were examined using light and scanning electron microscopy. Light microscopic examination followed the procedure described by Savile (30). Urediniospores were heated in a drop of lactophenol just to the boiling point and were studied to determine number and location of germ pores. Samples were used either fresh or after storage in liquid nitrogen; stored isolates were revived as described.

For examination using scanning electron microscopy, urediniospores were fixed in 4% gluteraldehyde for 4 hr and dehydrated in an ethyl alcohol series for at least one-half hour in each concentration from 10 to 100% (v/v) in 10% increments. Spores were resuspended in 100% ETOH and delivered to the USDA Systematic Botany and Mycology Laboratory in Beltsville, MD. Final preparation for scanning electron microscopy involved critical-point drying and sputter-coating with gold. Samples were examined for surface features, particularly hilum characteristics, with an Amray 1200B scanning electron microscope at ×3,000. Percent cover by the echinulations was measured for 25 urediniospores. Isolates of *C. pycnocephalus* from Italy, Greece, and Turkey, and isolates of *C. thoermeri* from Bulgaria, Greece, and Turkey were compared with the California isolate from *C. tenuiflorus*.

Measurements of urediniospore volume were made using an Elzone 80XY Particle Counter (Particle Data, Inc., Elmhurst, IL). Spores were heat-shocked upon recovery from liquid nitrogen, as described, and put into distilled water with 0.1% Tween 20, polyoxyethylene (20) sorbitan monolaurate, overnight to hydrate. The particle counter was calibrated for volume using latex beads. Three subsamples of at least 1,200 urediniospores were measured for each isolate, and values for mode of each subsample were used for statistical analysis.

RESULTS

Reciprocal inoculations. Distinct patterns of susceptibility emerged from inoculation of six *Carduus* species and artichoke with isolates of *P. carduorum* from *C. pycnocephalus*, *C. tenuiflorus*, and *C. thoermeri* (Tables 2 and 3). Results were the same whether infection type or disease severity data were analyzed. Ratings for both parameters were always higher for *C. thoermeri* inoculated with the various isolates from *C. thoermeri* than for reactions on other species. The mean IT rating of 3.5 for *C. thoermeri* was significantly higher ($P = 0.05$) than the mean rating of 1.7 for reactions on *C. tenuiflorus* (Table 2). The isolate, by host interaction, was statistically significant. Variation in both the magnitude of differences in ratings for each of the most susceptible hosts and the ranking of susceptibility of hosts to the various isolates was noted.

Ratings for both parameters were also significantly greater on *C. tenuiflorus* inoculated with the isolate from *C. tenuiflorus* (Table 3, isolate 84-03). Among the *Carduus* species, this isolate only attacked *C. tenuiflorus*; the mean IT rating for *C. tenuiflorus* was 4.0, but less than 0.1 for *C. thoermeri* and 0.0 for the other species.

A different pattern emerged from inoculation of the *Carduus* species with isolates from *C. pycnocephalus* (Table 3). Ratings for both parameters were always highest on *C. tenuiflorus* inoculated with these isolates, and the mean IT rating of 3.8 for *C. tenuiflorus* was significantly higher ($P = 0.05$) than mean ratings

TABLE 2. Mean values for infection type[a] (IT) from six *Carduus* species and *Cynara scolymus* (artichoke) inoculated with isolates of *Puccinia carduorum* from *Carduus thoermeri*

	Isolate number										
Species	78-02	78-03	78-04	78-05	78-08	78-09	84-32	84-40	84-87	84-88	Means[b]
Carduus											
acanthoides	0.0 d	0.0 d	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c	0.0 d	0.0 c	0.0 d
maculosa	2.0 b	0.8 c	1.2 b	0.0 c	1.0 b	0.8 b	0.0 c	0.0 c	1.0 c	0.0 c	0.7 c
nutans	0.6 cd	0.0 d	0.0 c	0.0 c	0.0 c	0.4 bc	0.0 c	0.0 c	0.0 d	0.8 bc	0.2 d
pycnocephalus	0.0 d	0.0 d	0.0 c	0.0 c	0.0 c	0.0 c	0.4 bc	0.0 c	0.0 d	0.0 c	+[c] d
tenuiflorus	2.0 b	1.4 b	0.2 c	1.8 b	1.6 b	1.1 b	0.7 b	3.8 a	2.0 b	2.0 a	1.7 b
thoermeri	3.8 a	3.9 a	4.0 a	3.6 a	4.0 a	1.2 a	3.4 a	4.0 a	3.4 a	2.4 a	3.5 a
Cynara											
scolymus	1.2 c	1.3 bc	1.4 b	2.0 b	1.8 b	1.0 b	0.8 b	2.8 b	1.0 c	1.6 ab	1.5 b

[a] IT is a rating for disease severity based upon pustule size, ranging from 0 (no macroscopic symptoms) to 4 (large pustules with abundant sporulation).
[b] Mean values followed by the same letter within columns (for each isolate) are not significantly different, according to Fisher's LSD ($P = 0.05$).
[c] + = Rating for IT is less than 0.06.

TABLE 3. Mean values for infection type[a] (IT) from six *Carduus* species and *Cynara scolymus* (artichoke) inoculated with isolates of *Puccinia carduorum* from *Carduus pycnocephalus* and *C. tenuiflorus*

	Source of isolate/isolate no.									
	C. pycnocephalus									*C. tenuiflorus*
Species	84-06	84-07	84-58	84-83	85-18	85-80	85-128	85-160	Means[b]	84-03
Carduus										
acanthoides	0.0 c	0.0 b	0.0 b	0.0 c	0.0 d	0.0 d	0.0 c	0.0 c	0.0 c	0.0 c
maculosa	0.0 c	0.0 b	0.0 b	0.0 c	0.0 d	0.0 d	0.0 c	0.0 c	0.0 c	0.0 c
nutans	0.0 c	0.0 b	0.0 b	0.0 c	0.0 d	0.4 cd	0.0 c	0.0 c	+[c] d	0.0 c
pycnocephalus	0.4 c	0.8 b	3.2 a	2.2 b	2.6 b	2.4 b	1.6 a	3.2 a	2.0 b	0.0 c
tenuiflorus	4.0 a	4.0 a	4.0 a	3.8 a	4.0 a	3.4 a	2.2 a	4.0 a	3.8 a	4.0 a
thoermeri	0.0 c	0.0 b	0.0 b	0.0 c	0.0 d	1.0 c	0.0 c	0.0 c	0.1 d	+ c
Cynara										
scolymus	1.9 b	0.0 b	4.0 a	0.0 c	0.9 c	2.2 b	1.8 a	2.6 b	1.7 c	2.0 b

[a] IT is a rating for disease severity based upon pustule size, ranging from 0 (no macroscopic symptoms) to 4 (large pustules with abundant sporulation).
[b] Mean values followed by the same letter within columns (for each isolate) are not significantly different, according to Fisher's LSD ($P = 0.05$).
[c] + = Rating for IT is less than 0.06.

on *C. pycnocephalus* (2.0) or *C. nutans* and *C. thoermeri* (0.1 or less). Host interaction with the isolate was also significant in this analysis, and variation was noted only in the magnitude of differences between mean ratings for each of the most susceptible hosts.

Artichoke was moderately susceptible to isolates from all three hosts (Tables 2 and 3), and the mean IT ratings for *C. pycnocephalus*, *C. tenuiflorus*, and *C. thoermeri* were 1.7, 2.0, and 1.5, respectively. Susceptibility of artichoke did not differ by isolate source.

The isolate of *P. carthami* from safflower did not infect any of the *Carduus* species in this test, and safflower was not susceptible to any of the rust isolates from *Carduus* (data not shown).

In a separate study, collections of *C. pycnocephalus* from California differed in susceptibility to rust from *C. pycnocephalus* (Table 4). Collection number 27, with a mean IT rating of 2.1, was significantly more susceptible ($P = 0.05$) to all the isolates in the test than was collection number 29. Both of these collections were more susceptible than the other collections, the latter set being susceptible only to isolate 85-18. Ratings for disease severity and infection type were not particularly high in this study, even on the most susceptible collections of *C. pycnocephalus* from California.

Morphological determinations. Urediniospores typical of *P. carduorum* were observed in this study, including the small verrucose hilum considered characteristic of the *P. centaureae–P. laschii* lineage (28). Features of the hilum were particularly clear with scanning electron microscopy.

Morphological distinctions were not readily apparent on the basis of bright-field microscopic examination. However, the isolates could be grouped by host plant on the basis of urediniospore volume and by coverage of spores by echinulations (Table 5). Urediniospores of isolates from *C. pycnocephalus* were 16% larger and had 9.9% more of the surface covered by echinulations than those from *C. thoermeri*. Similarly, the isolate from *C. tenuiflorus* was 12% larger and had 12.7% more of the surface covered by echinulations than isolates from *C. thoermeri*. Differences in each of the above cases were statistically significant ($P = 0.05$), but comparisons of volume and coverage of urediniospores between *C. tenuiflorus* and *C. pycnocephalus* were not significantly different. The mean volume of *P. carthami* uredindiospores was 4,195 μ^3, which was not different from *P. thoermeri*.

Isozyme analysis. Fifteen enzymes were resolved sufficiently to allow interpretation of 17 putative isozyme loci for isolates of *P. carduorum* in this study. Of these enzymes, GPI, GK, and AAT separated the pathogen by host species. Isozyme banding patterns for isolates from *C. pycnocephalus* were identical (CS = 1.00), regardless of geographic source (Table 6), as were isolates from *C. thoermeri*, also representing different locations. The CS value, comparing nine isolates from *C. pycnocephalus* with seven isolates from *C. thoermeri*, was 0.89, the lowest intergroup CS for isolates from *Carduus*. Values comparing the groups of CS isolates from the three *Carduus* species with the isolate of *P. carthami* from safflower ranged from 0.50 to 0.60. Also, isolates of *P. carduorum* could be differentiated from the *P. carthami* isolate based on migration of variants of enzymes MDH and MPI.

Putative alleles for the enzymes AAT, GK, and GPI are presented in Table 7. Three putative loci were discovered for AAT and designated (in order of decreasing distance of zone of enzymatic activity from the origin) as Aat1, Aat2, and Aat3, respectively. All isolates showed Aatl-100, but only isolates from *C. thoermeri* displayed activity at loci Aat2 and Aat3. Based on a lack of enzymatic activity, null alleles were postulated for Aat2 and Aat3 for isolates from *C. pycnocephalus*, *C. tenuiflorus*, and safflower.

All isolates from *C. pycnocephalus* were genotypically Gk-100. Isolates from *C. thoermeri* and safflower expressed allele Gk-115, and the isolate from *C. tenuiflorus* displayed allele Gk-69.

All isolates from the *Carduus* species, with one exception, possessed allele Gpi-100, including two Italian isolates from *C. pycnocephalus* (84-06 and 85-80). Another Italian isolate from *C. pycnocephalus* (85-128) and the isolate from *P. carthami* expressed Gpi-125.

One isolate was increased in both artichoke and its source plant, *C. pycnocephalus*. No isozyme differences were detected between inoculum from artichoke and *C. pycnocephalus*.

TABLE 4. Mean values for infection type[a] (IT) from California *Carduus pycnocephalus* collections inoculated with *Puccinia carduorum*

C. pycnocephalus collection no.	Isolate				Means[b]
	84-07	84-23	85-18	85-80	
24	0.0 c	0.0 c	0.4 cd	0.0 b	0.1 c
25	0.0 c	0.0 c	0.2 d	0.0 b	0.1 c
26	0.0 c	0.0 c	0.7 c	0.0 b	0.1 c
27	1.3 b	2.1 a	3.5 a	1.5 a	2.1 a
28	0.0 c	0.0 c	0.4 cd	0.0 b	0.1 c
29	2.0 a	1.0 b	3.0 b	1.1 b	1.8 b
30	0.0 c	0.0 c	0.2 d	0.0 b	0.1 c
Means[b]	0.48 b	0.44 b	1.22 a	0.38 b	

[a] IT = Rating for infection type. Values for IT range from 0 (no macroscopic symptoms) to 4 (large, often coalescing pustules).
[b] Means followed by the same letter are not significantly different, according to Fischer's LSD ($P = 0.05$).

TABLE 5. Least square means for two morphological features of urediniospores of *Puccinia carduorum* from *Carduus pycnocephalus*, *C. tenuiflorus*, and *C. thoermeri*

Host species	Urediniospore volume		Echinulation cover	
	No. of isolates	μ^3	No. of isolates	%
Carduus				
pycnocephalus	7	4,987 a[z]	3	75.4 a
tenuiflorus	1	4,778 a	1	78.2 a
thoermeri	8	4,182 b	3	65.5 b

[z] Means followed by the same letter in each column are not significantly different, according to Fisher's LSD ($P = 0.05$).

DISCUSSION

Rust isolates from *C. pycnocephalus*, *C. tenuiflorus*, and *C. thoermeri* clearly fit Savile's description for *P. carduorum*, which is based on 15 isolates from *Carduus crispus* L. (28). Data from the isozyme analysis, in particular, support conclusions of Savile (28), Panditou (22), and Urban (34) that these are the same species, and that *Puccinia cardui-pycnocephali* Syd. is synonymous with

TABLE 6. Rogers' Coefficient of Similarity (CS) for isozyme banding patterns of rust isolates from *Carduus pycnocephalus*, *C. tenuiflorus*, *C. thoermeri*, and *Carthamus tinctorius* (safflower)

Source host	No. of isolates	*Carduus*			*C. tinctorius*
		pycnocephalus	*tenuiflorus*	*thoermeri*	
C. pycnocephalus	9	1.00	0.90	0.89	0.55
C. tenuiflorus	1		...[a]	0.95	0.60
C. thoermeri	9			1.00	0.50
C. tinctorius	1				...

[a] No comparison, only one isolate.

P. carduorum. Based on isozyme analysis, all isolates of *P. carduorum* are closely related. CS values for each pair of isolates of *P. carduorum* ranged from 0.82 to 0.93, in contrast to a range of 0.50–0.60 when comparing isolates of *P. carduorum* with *P. carthami*. The latter falls below the limit of 0.70 proposed by Gottlieb (11) for intraspecial variation.

Differences were evident among isolates grouped by *Carduus* species on the basis of isozyme analysis, host range, urediniospore volume, and coverage by echinulations. Differences were also reported for specimens of *P. carduorum* examined by Savile (28), who described morphological differences between teliospores of *P. carduorum* from *Carduus defloratus* L. and *C. crispus*. Savile also reported that two specimens from *C. acanthoides* differed from *C. crispus* specimens in coverage of urediniospores by echinulations. Six rusts of *C. pycnocephalus* from Spain, Italy, and Greece in this study also were "very close to *P. carduorum*," and five of these isolates showed "substantial unity in certain characters." Savile concluded, "it appears best to regard *P. carduipycnocephali* as a variety of *P. carduorum* with some adaptation to arid summers."

Morphological differences between isolates of *P. carduorum* from different *Carduus* hosts were found, but the study is limited by the number of *Carduus* hosts and, for *C. tenuiflorus*, by the number of isolates. The fact that these differences exist indicates that a more detailed study is needed, particularly one that includes members of the *P. centaureae–P. laschii* lineage (28) from hosts other than *Carduus*. Classification of the rusts of *Carduus* at the subspecific level may be possible by using urediniospore and teliospore morphology in combination with data on host specificity and isozyme banding patterns.

Although the number of isolates from *C. pycnocephalus* and *C. thoermeri* were limited, these data are representative of the geographical range encompassed by the isolates. Additional isolates from *C. tenuiflorus* need to be examined to accurately determine how they relate taxonomically to isolates from *C. pycnocephalus* and *C. thoermeri*. The preliminary data suggest a very close relationship between isolates from *C. tenuiflorus* and *C. pycnocephalus*, which may reflect a very close relationship between the host plants.

Morphological features of urediniospores observed in this study and by others do not seem practical for identification of these fungi to subspecies or even to species. Even though isolates from *C. pycnocephalus* and *C. thoermeri* were different based on urediniospore volume and coverage by echinulations, the *C. tenuiflorus* isolate was not different from *C. pycnocephalus* isolates in either case.

California populations of *C. pycnocephalus* differed greatly in their susceptibility to isolates of *C. pycnocephalus* rust. This was different from results with *P. carduorum* from *C. thoermeri*, as all collections of *C. thoermeri* from North America were uniformly susceptible to *P. carduorum* in an earlier study (24). Specificity within host species has been a factor in the success of *P. chondrillina* Bubák & Syd. for control of *Chondrilla juncea* L. (rush skeleton weed) in Australia (6). Three morphological types of skeleton weed occur in Australia, but only one, the most common type, is susceptible to the strain of *P. chondrillina* introduced for biological control. This has resulted in significant reduction in density of the susceptible type and economic justification for the program (6). However, populations of the other types are increasing, and additional strains of the pathogen are sought.

The relatively greater susceptibility of *C. tenuiflorus* to *P. carduorum* from *C. pycnocephalus* may have implications in biological weed control. Savile (28) speculated that *P. carduorum* from *C. pycnocephalus* may be "confined to *C. pycnocephalus*." Susceptibility of *C. tenuiflorus* to isolates from *C. pycnocephalus* suggests either that a lack of homeostasis exists between *C. tenuiflorus* and isolates from *C. pycnocephalus*, or that the host species are very closely related. The concept of homeostasis and biological control of weeds with insects has been discussed (7). Data in this study suggest this possibility exists for plant pathogens and biological control of weeds. Search for an isolate from *C. tenuiflorus* that is more aggressive on *C. pycnocephalus* than the current acquisitions may be justified on this basis. The geographical ranges of *C. tenuiflorus* and *C. pycnocephalus* are reported to overlap only in Italy (33), where *C. pycnocephalus* is much more common than *C. tenuiflorus* (23). The range of *C. pycnocephalus* includes Bulgaria, Greece, Italy, Romania, and Turkey. *C. tenuiflorus* is found in Western Europe. We are interested in acquiring Western European isolates of rust of *C. tenuiflorus* for reciprocal inoculations.

Our data suggest that isozyme analysis may be useful for positive identification of species in the *Puccinia centaureae–P. laschii* lineage (28) in the absence of host plant information. Each isolate from *C. pycnocephalus* and *C. thoermeri* was identical to the others from its respective host, but there were slight differences between isolates from the different *Carduus* species. Additional study, including rust isolates from other species in the Cardueae, will be needed to develop the necessary database to make this practical.

The ability to positively identify new acquisitions of *P. carduorum* by using isozyme analysis or other procedures will enable predictions to be made regarding expected nontarget reactions, such as the susceptibility of artichoke and *Cirsium* species. Also, with the accumulation of field data on host specificity and performance by isolates of *P. carduorum* expected to be approved for release, positive identification of new acquisitions will support predictions on field performance and potential for biological control.

TABLE 7. The relative mobility of putative alleles associated with three enzymes of isolates from *Carduus pycnocephalus*, *C. tenuiflorus*, *C. thoermeri*, and *Carthamus tinctorius* (safflower)

	Source of isolate			
	Carduus			*Carthamus*
Enzyme[a]	*pycnocephalus*	*tenuiflorus*	*thoermeri*	*tinctorius*
AAT	Aat1-100	Aat1-100	Aat1-100	Aat1-100
	Aat2-null	Aat2-null	Aat2-100	Aat2-null
	Aat3-null	Aat3-null	Aat3-100	Aat3-null
GK	Gk-100	Gk-69	Gk-115	Gk-115
GPI	Gpi-100	Gpi-100	Gpi-100	Gpi-125
	Gpi-125[b]			

[a] AAT = Aspartate aminotransferase, GK = glukokinase, and GPI = glucose phosphate isomerase.

[b] For one of three isolates from *C. pycnocephalus* (85-128), the putative allele was assigned a value of Gpi-125.

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