Susceptibility of Musk Thistle and Related Composites to *Puccinia carduorum*

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ABSTRACT

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Evaluation of the rust fungus *Puccinia carduorum* for biological control of musk thistle (*Carduus nutans*) was initiated with a host range study, the first phase in determining the potential and safety of an exotic plant pathogen for use in biological weed control. Representatives of the 13 tribes in the Asteraceae (26 genera and 63 species) were tested for susceptibility to *P. carduorum*. Only members of the subtribe Carduineae in the tribe Cynareae were susceptible under greenhouse conditions. Most collections of musk thistle from the United States, Canada, and France were highly susceptible. *Carduus thoermeri* and *Cynara scolymus* (globe artichoke) also were susceptible to infection. Pustules occurred on six additional species of *Carduus*, eight *Cirsium* spp. and *Cynara cardunculus*. Seedlings of globe artichoke were much more susceptible to infection than older plants grown from seed or crowns. Further work, now in progress, will determine the impact of *P. carduorum* on globe artichoke, the only species of economic importance found susceptible to the fungus under greenhouse conditions.

Several *Carduus* species introduced into North America have become aggressive weeds of pastures, ranges, roadsides, and disturbed areas. The genus was reviewed recently in Flora Europaea (5), but the full complement and distribution of *Carduus* species in North America has not yet been determined. McCarty (13,14) reported that seven *Carduus* species occur in the United States: *C. macrocephalus* Desf., *C. nutans* L., *C. thoermeri* Weinm. (large-flowered species), *C. acanthoides* L., *C. crispus* L. (small-flowered species), *C. pycnocephalus* L., and *C. tenuiflorus* Curt. (slender-flowered species). Moore and Frankton (16) classify the large-flowered *Carduus* as one species, *C. nutans*, composed of three subspecies.

The large-flowered species, identified as *C. nutans* ssp. *leiophyllus* (Petrovic) Stoj. & Stef. (16) (referred to as *C. nutans* in this paper), is widespread in the United States (3). The remaining weedy *Carduus* species have limited distributions, with *C. acanthoides* a problem only in Virginia and parts of the midwest, and *C. pycnocephalus* and *C. tenuiflorus* in California (3,4).

Since these weedy *Carduus* species occur commonly in pastures and rangelands, control with herbicides or cultural practices is generally not feasible, primarily for economic reasons. Therefore, biological control with introduced natural enemies is being studied (1,10,21). Two insects have been released in Canada and the United States for biocontrol of *Carduus*. A seed-head weevil, *Rhinocyllus conicus* Froelich, is established on *C. acanthoides, C. nutans,* and *C. pycnocephalus*, and a meristem (rosette) weevil, *Trichosirocalus horridus* (Panzer) [*Ceuthorhynchidius horridus* (Panzer)], is established on *C. nutans* (11,12). *R. conicus* has effectively reduced the density of *C. nutans* in some areas, but additional biological control agents are required. Among those biological control agents being considered are plant pathogens collected in Eurasia.

At least four *Puccinia* species, including *P. carduorum* Jacky, *P. cardui-pycnocephali* Syd., *P. galatica* Syd., and *P. hadacii* Urban, have been reported to attack various *Carduus* species in Eurasia (19). None of these rust fungi was thought to occur in North America (2,20), but *P. carduorum* recently was identified on *C.*

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tenuiflorus Curt. in California (A. K. Watson, *unpublished*). Currently, isolates of *P. carduorum* collected in Eurasia are being evaluated for biological control of *C. nutans* in the United States and Canada.

The objective of this study was to determine the host specificity of these isolates. Some of these results were reported earlier (17,18).

MATERIALS AND METHODS

Rust collection. Isolates of *P. carduorum* were collected from *C. nutans* by R. G. Emge in Bulgaria, Romania, and Turkey in 1978; transported to Frederick, MD; increased in the disease containment facility at the Plant Disease Research Laboratory (PDRL); and stored either as urediniospores on infected leaf material, or in ampules in a liquid nitrogen refrigerator. Isolates were revived after freezing by heat shocking (40 C for 2 min), hydrating, and inoculating *C. nutans* in containment.

Plants tested. The plant species used in the present study were selected from within the framework of the centrifugal phylogenetic testing sequence (26). In this system, the intensity of testing is greatest on the target species (*C. nutans*) and related taxa, and taxa of increasingly distant relationship are tested to a lesser degree. Thus, many collections of the target weed from the United States, Canada, and Europe were tested along with several other *Carduus* species, other members of the Cynareae tribe, and representatives of the remaining tribes in the Asteraceae.

Plant propagation. Seeds of species tested at PDRL, except for globe artichoke (*Cynara scolymus* L.), were treated overnight with gibberelic acid (10^{-3} M) and sown in 10-cm-diameter clay pots containing a pasteurized mix of field soil, sand, and peat moss (2:1:1, v/v). Plants were grown for 4–5 wk in the containment greenhouse before inoculation. Globe artichoke crowns were split and grown in 15-cm-diameter plastic pots filled with the pasteurized mix and sand (1:1, v/v) for 3–6 mo before inoculation. Temperature in the greenhouse was maintained between 21 and 25 C and lighting was natural except in winter when cool-white fluorescent lights were used to supplement natural light and extend day length to 12 hr. Plants were watered regularly to maintain healthy, vigorous growth.

At Macdonald College, test plant material was grown from seed in prepared soil mix (PROMIX) with one to four plants per 10-cm-diameter plastic pot. Plant material was maintained in controlled environment chambers at 15 C for 10 hr in the dark, and 20 C for 14 hr in the light (237 $\mu E/m^2/sec$). Plants were watered regularly and fertilized as required to maintain healthy growth.

Inoculations. Three isolates of *P. carduorum* (III and IV from Turkey and VII from Romania) were maintained at PDRL on *C. nutans* in separate cubicles in the containment greenhouse facility, and fresh inoculum from these source plants was used for most studies. Occasionally, material that had been stored in liquid nitrogen was used for inoculation. The isolate used for most of the study at PDRL was collected near Ankara, Turkey. An isolate from a different plant at the same location in Turkey was obtained from PDRL and studied in the Macdonald College quarantine facility.

Plants were inoculated either by spraying to runoff with a suspension of urediniospores at 1 mg/20 ml in water (plus one drop of Tween-20 per 50 ml) or by use of a turntable settling tower (15) with 1 mg of urediniospores for each inoculation (eight plants). Spraying was used for routine maintenance of inoculum; the settling tower was used when uniform inoculation and quantification of infection were desired. Plants were inoculated 5–7 wk after seed planting.

At Macdonald College, inoculum was maintained on *C. nutans*, and test plants were generally inoculated between the second- and sixth-leaf stage of growth. Occasionally older plant material was inoculated. Test plants were inoculated by: a) hydrating urediniospores for 3 hr by placing small leaf pieces with uredinia into a moist chamber, b) moistening plants with a 0.5% gelatin solution in distilled water applied with an atomizer spray bottle, c) transferring a small amount of hydrated urediniospores to the leaf surface, d) spreading the urediniospores over the leaf surfaces lightly by hand, e) lightly spraying inoculated plants with distilled water and enclosing the plants in a plastic bag, and f) maintaining inoculated plants in this humid environment for 12 hr in the dark. Inoculated plants then were returned to the growth chambers.

Disease ratings. A rating scheme similar to that used for description of wheat stem rust pustules was used in this study. The rating symbols and their descriptions are as follows:

Infection type	Description of reaction
0	Immune; no symptoms.
0	Hypersensitive; chlorotic halo, no uredinia.

TABLE 1. Reaction of species of Asteraceae inoculated with *Puccinia carduorum*, isolate III[a] at Frederick, MD

Tribe, species, and cultivar	Infection type (0–4)[b]
Anthemideae	
Chrysanthemum carinatum Schousb. 'Rainbow'	0
C. indicum L. 'Korean'	0
C. maximum Ramond 'Alaska'	0
Arctotideae	
Gazania splendens grandiflora L. 'Sunshine'	0
Astereae	
Callistephus chinensis (L.) Nees 'American Beauty Aster'	0
C. chinensis 'Dwarf Border Aster'	0
Calenduleae	
Calendula arvensis L.	0[c]
C. officinalis L.	0
Cichorieae	
Cichorium endivia L.	0
C. intybus L.	0[c]
C. pumilum Jacq.	0
Chondrilla juncea L.	0
Lactuca sativa L. 'New York Special'	0
Cynareae (=Cardueae)	
Subtribe Carduinae	
Arctium lappa L.	0
Carduus acanthoides L.	0
C. nutans L.	0 to 4[d]
C. pycnocephalus L.	0
C. tenuiflorus Curt.	1
Cirsium altissimum (L.) Spreng.	0
C. andersonii (Gray) Petr.	2 to 3
C. andrewsii (Gray) Jeps.	0
C. arizonicum (Gray) Petr.	0 to 2
C. brevistylum Cronq.	0
C. callilepsis (Greene) J. T. Howell	2 to 3
C. campylon H. K. Sharsm	0
C. cymosum (Greene) J. T. Howell	0 to 3
C. discolor (Muhl.) Spreng.	0
C. douglasii DC.	0
C. occidentale (Nutt.) Jeps.	2 to 3
C. ochrocentrum Gray	0
C. pastoris J. T. Howell	2 to 4
C. proteanum Howell	2 to 3
C. flodmanii (Rydb.) Arthur	0
C. vulgare (Savi) Ten.	0 to 3
Cynara cardunculus L.	2 to 3
C. scolymus L. 'Green Globe'	2 to 3
Subtribe Centaureinae	
Carthamus tinctorius L. 'S-208'	0
Centaurea cineraria L.	0
C. solstitialis L.	0
C. vira-vira L. 'Candidissima'	0
Cnicus benedictus L.	0
Cyanus segetum (L.) Hill 'Batchelor's Button'	0
Eupatorieae	
Ageratum houstonianum Mill. 'Blue Mink'	0
A. houstonianum 'Spindrift hybrid'	0
Helenieae	
Helenium microcephalum DC.	0

(continued)

TABLE 1 (continued).

Tribe, species, and cultivar	Infection type (0–4)[b]
Heliantheae	
Cosmos sulphureus Cav.	0
Dahlia pinnata Cav.	0
Helianthus annuus L. 'Mammoth'	0
Tagetes patula L. 'Orange Hawaii'	0
T. patula 'Brownie Scout'	0
T. patula 'Petite Yellow'	0
Zinnia elegans Jacq. 'Giant Cactus Flower'	0
Z. elegans 'Giant Flowers'	0
Innuleae	
Helichrysum bracteatum Ndr.	0
Mutisieae	
Gerbera jamesonii J. Bolns ex Hook. 'Transvaal Daisy'	0
Senecioneae	
Senecio cineraria DC. 'Maritima'	0
S. cineraria 'Silver Dust'	0
Vernonieae	
Vernonia anthelmitica (L.) Willd.	0[c]

[a] Plants in four to six pots (4–10 plants per pot) were tested for each entry unless otherwise indicated.
[b] Disease scale of 0 to 4, with 0 = no symptoms and 4 = highly susceptible.
[c] Two different collections inoculated.
[d] Twenty-seven collections inoculated. See Table 3 for more details.

TABLE 2. Responses of plants inoculated with *Puccinia carduorum* isolate III, at Macdonald College, McGill University, Ste-Anne-de Bellevue, Quebec, Canada

Tribe, subtribe, and species	Plants inoculated (no.)	Infection type (0–4)[a]
Cynareae (Cardueae)		
Carduinae		
Carduus nutans L.		
Saskatchewan[b]	32	2 to 4
France	1	2
Ontario	1	0
Carduus acanthoides L.	2	0 to 0;
Carduus carlinoides Gouan	3	0 to 2
Carduus crispus L.	8	0; and 1
Carduus defloratus L.	11	0 to 1
Carduus macrocephalus Desf.	5	0; to 2
Carduus personata (L.) Jacq.	7	0;
Carduus pycnocephalus L.	10	0
Carduus squarrosus (DC.) Lowe	5	0 to 2
Carduus tenuiflorus Curt.	2	0;
Carduus thoermeri Weinm.[c]	9	2 and 3
Cirsium arvense (L.) Scop.	4	0
Cirsium vulgare (Savi) Ten.	2	0
Cynara cardunculus L.	1	0
Cynara scolymus L.		
'Green Globe'	7	2 and 3
Centaureinae		
Carthamus lanatus L.	12	0;
Carthamus tinctorius L.		
'Dart'	4	0;
'Gila'	2	0
'UC-41'	2	0
'PCOY'	1	0
'PCM-1'	6	0
'PCM-2'	8	0
'PCN'	5	0
'Reduced Hull'	4	0;
Hudson Seeds	12	0
Centaurea diffusa Lam.	3	0
Centaurea jacea L.	2	0
Centaurea maculosa Lam.	2	0
Centaurea nigra L.	2	0

[a] Rating system used: Disease scale of 0 to 4, with 0 = no symptoms and 4 = highly susceptible.
[b] Location of seed source.
[c] According to Moore and Frankton (16), *C. thoermeri* is considered synonymous with *C. nutans* ssp. *leiophyllus*.

Infection type	Description of reaction (continued)
1	Very resistant; minute uredinia surrounded by necrotic area.
2	Moderately resistant; small to medium sized uredinia.
3	Moderately susceptible; medium sized uredinia, no necrosis, may be some chlorosis.
4	Very susceptible; large uredinia often coalescing, no necrosis, possibly chlorosis.

RESULTS

The host range of *P. carduorum* tested in these studies was restricted to the subtribe Carduinae of the tribe Cynareae (Cardueae) in the Asteraceae (Tables 1 and 2). Most collections (23 of 27 tested at Frederick, MD) of *Carduus nutans* were very susceptible to the fungus (Table 3). Young globe artichoke plants (*Cynara scolymus*), *Cynara cardunculus*, and individuals of eight *Cirsium* species (*C. andersonii, C. arizonicum, C. callilepsis, C. cymosum, C. occidentale, C. pastoris, C. proteanum*, and *C. vulgare*) were susceptible (infection types 1–4) to the rust fungus. Attempts to reinoculate the *Cirsium* spp. were unsuccessful in most cases. Small pustules (infection types 1–2) were noted also on individuals of *Carduus carlinoides, C. crispus, C. defloratus, C. macrocephalus, C. squarrosus*, and *C. tenuiflorus*.

Twenty-seven collections of *C. nutans* tested against *P. carduorum* at PDRL (Table 3) were susceptible to isolate III except

TABLE 3. Susceptibility of different collections of *Carduus nutans* at Frederick, MD, to three isolates of *Puccinia carduorum*

Seed source	Designation[a]	Isolates[b] III	IV	VII
British Columbia	M-24-5	13/13[c]	...[d]	2/3
	M-24-9	10/10	...	...
	M-24-10-0	13/13	...	2/2
	M-24-10-S	13/13	...	2/2
	Alexis Creek	14/14	...	...
California	Mt. Shasta	14/14	...	...
France	M-26-9	0/15	...	...
Illinois	M-11-1	17/18	3/3	3/3
Iowa	M-23-5	22/22	3/3	3/3
Kentucky	M-14-2	18/18	3/3	3/3
Maryland	M-25-1	0/13	...	0/2
	M-25-2	1/13	...	0/2
	Frederick	30/30	3/3	3/3
	Sparks	12/12	12/12	12/12
Mississippi	M-17-2	13/13	...	2/2
Missouri	M-12-2	27/27	3/3	3/3
Montana	M-27-5	16/18	...	2/2
	M-27-11	13/13	...	2/2
	M-50-5	0/17	...	1/2
	M-53-2	11/18	...	2/2
	M-53-6	17/18	...	2/2
Nebraska	M-19-2	21/22	3/3	3/3
	Nebr.	14/14	...	...
Saskatchewan	M-16-1	18/18	3/3	3/3
	Sask.	14/14	...	...
Wisconsin	Wisc.	14/14	...	...
Virginia	M-21-5	22/22	3/3	3/3

[a] M = seed from M. K. McCarty; remaining designations are seeds from collections at PDRL when the present study initiated.
[b] Isolates III and IV were from Turkey, and isolate VII was from Romania.
[c] Number of infected plants/number of inoculated plants.
[d] ... = Not tested.

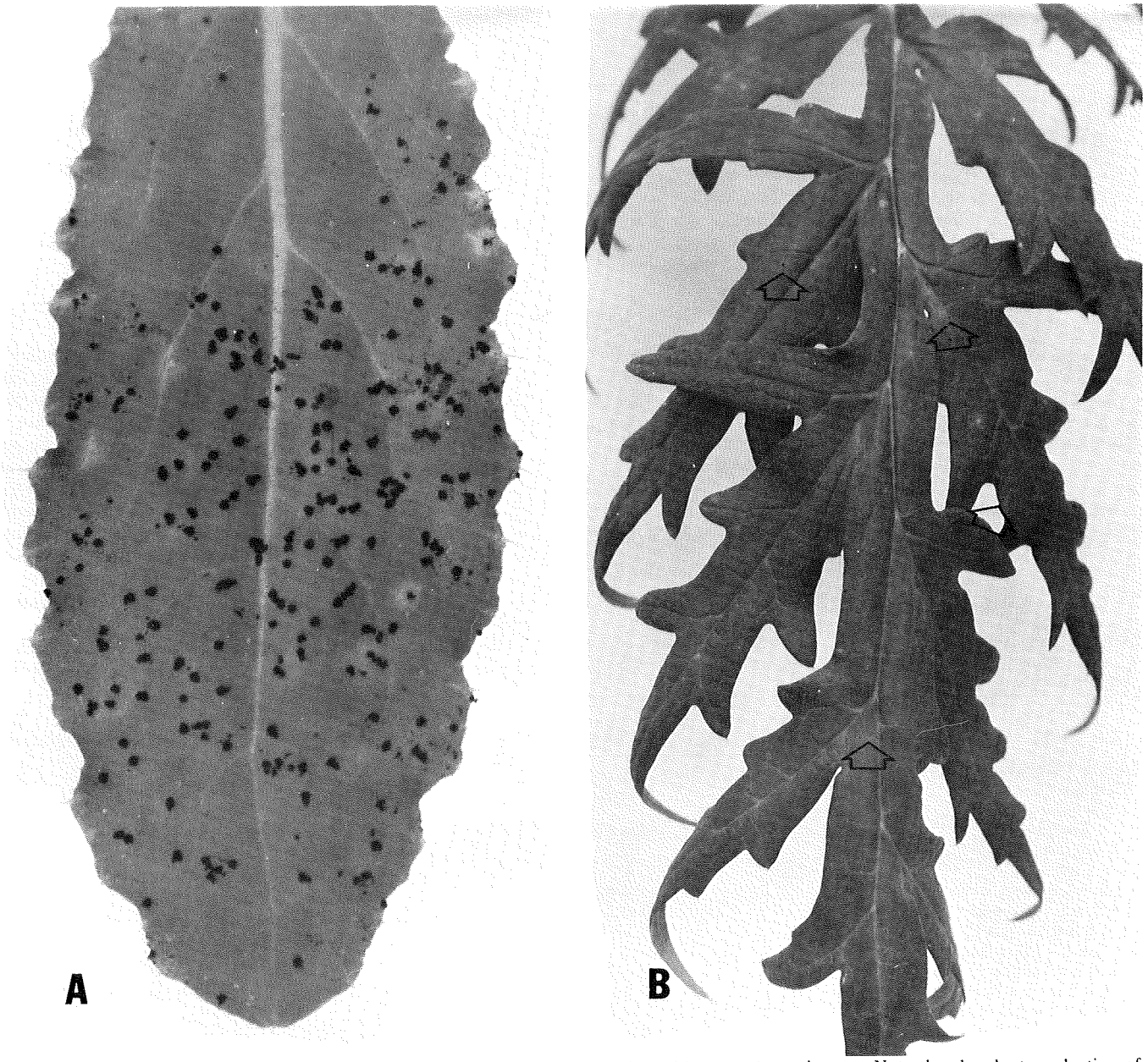

Fig. 1. Leaves of **A,** *Carduus nutans* and **B,** *Cynara scolymus* 2 wk after inoculation with *Puccinia carduorum.* Note the abundant production of urediniospores on the leaf of *C. nutans.* Pustules are barely visible on the leaf of *C. scolymus* (arrows).

two from Maryland (25-1, 25-2), one from Montana (50-5), and one from France (26-9). In another plant collection from Montana (53-2), 65% of the plants were susceptible to *P. carduorum.* Two other isolates of *P. carduorum* (IV and VII) caused similar reactions as III when used to inoculate a limited number of collections of *C. nutans* (Table 3).

Symptomatology. When musk thistle was inoculated with urediniospores of *P. carduorum* under greenhouse conditions, white blisterlike flecks appeared within 6–7 days. The flecks developed into brown pustules 7–9 days after inoculation, and within 2 wk the fungus produced abundant urediniospores (Fig. 1A). Infected leaves turned yellow and died within 18–20 days after inoculation. The rust infection was not systemic. Globe artichokes grown from crowns and inoculated with urediniospores of *P. carduorum* developed in 12–14 days, and pustules appeared only on the lower, senescing leaves (Fig. 1B). Globe artichokes grown from seed (second- to sixth-leaf stage), and inoculated at Macdonald, were more susceptible to *P. carduorum* (infection types 2–3) than older plants from seed. Infected leaves of seedlings also became necrotic about 3 wk after inoculation.

DISCUSSION

Two isolates of *P. carduorum* from Turkey and one from Romania were very aggressive on most North American collections of *C. nutans*, and were virulent also on six (of ten) other *Carduus* species, two (of two) *Cynara* species, and eight (of sixteen) *Cirsium* species; all these genera belong to the subtribe Carduinae of the tribe Cynareae (Cardueae). None of ten additional species in the subtribe Centaureinae (tribe Cynareae) or 22 species in the 12 other tribes of the Asteraceae was susceptible to *P. carduorum.* These results indicate that the host range of *P. carduorum* isolates tested in this study is limited to the subtribe Carduinae in the tribe Cynareae and generally support the findings of Jacky (9) who reported that *P. carduorum* was confined to the genus *Carduus.* Jacky's report is further supported by the findings herein that only young plants in the genera *Cirsium* and *Cynara* were susceptible to the rust fungus, and inoculations of older plants (*Cynara scolymus*) or reinoculation of infected plants (*Cirsium* spp.) were either unsuccessful or resulted in much less aggressive infections.

Special consideration was given to globe artichoke in this study, because of its economic importance in California and parts of the

southern United States (8,22). To our knowledge, *P. carduorum* is not a pathogen of globe artichoke under field conditions in Eurasia, because it has never been reported on artichokes where artichokes, musk thistle, and *P. carduorum* are sympatric (2,6,7,19,20,23–25). It is possible that the limited susceptibility of artichoke to *P. carduorum* reported here is a greenhouse phenomenon. Further studies are in progress at PDRL and in Europe (field studies) to determine the impact of infection caused by *P. carduorum* on artichoke.

Not all North American collections of *C. nutans* were susceptible to the fungus, indicating that biotypes or taxa of the large-flowered *Carduus* species exist in North America which may not be attacked by the *P. carduorum* isolates examined in this study. A more complete understanding of the taxonomy of *C. nutans* in North America will help in determining a strategy for using *P. carduorum*. The number of resistant collections of *C. nutans* is limited, but the area these types infest in North America is unknown.

The aggressiveness of *P. carduorum* on *C. nutans*, and the limited susceptibility of other plant species indicate that *P. carduorum* has potential in biological control of musk thistle. Continuing studies on the stress caused by *P. carduorum* in nontarget suscepts will result in a clear understanding of the potential risk of disseminating this rust fungus in North America.

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