Population Dynamics and Spread of *Puccinia carduorum* in the Eastern United States

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ABSTRACT

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Puccinia carduorum was first introduced into western Virginia in 1987 for biological control of musk thistle (*Carduus thoermeri*). The pathogen's distribution was surveyed in 1992; it had spread to South Carolina, Georgia, Tennessee, Kentucky, Ohio, southeast Indiana, Maryland, and Delaware, some points more than 500 km from the release site. In 1994, it was found west of the Mississippi River in north central Missouri. Population development of the rust was monitored in several natural musk thistle stands in 1991 and 1992. Average pustule numbers per leaf were 0 to 0.7 in early May during early stem elongation, 0 to 17 (1991) and 8 to 52 (1992) at seed ripening in late June and early July as old plants died, 0.2 to 2.5 on young rosettes in September and October, and declining to near 0 by early December. Germinability of urediniospores from green tissue ranged from 10 to 88% (mean 51%) from May to October, with no significant seasonal trend. Teliospores were present on dead plants in late July and August but did not become prevalent on young rosettes until October and November. Latent periods (days from inoculation to first open pustule) on plants in the field were 13 to 14 days for inoculations in late April and early May, 8 days in June, 17 days in early October, and about 25 days in late October.

Puccinia carduorum Jacky is an autoecious rust fungus from Eurasia that attacks several species in the genus *Carduus*, including the musk thistle that is most prevalent in the United States (*Carduus thoermeri* Weinmann = *Carduus nutans* L. subsp. *leiophyllus* (Petrovic) Stoj. & Stef.) (5,12). It has been considered a potential biological control agent for this weed (15,16). After evaluation of this pathogen in a containment greenhouse at Frederick, Maryland, approval for a field test in the United States was granted in 1987 by the USDA Animal and Plant Health Inspection Service (APHIS) (3). The test site was near Blacksburg, Virginia, in an area with few musk thistle stands. This was done to reduce the risk of early extensive spread and to facilitate eradication if, on the basis of the first year's results, the pathogen was deemed too hazardous to be established permanently.

The pathogen was introduced into field plots in October 1987, and several additional inoculations were performed in 1988 through the spring of 1990. These experiments (3) established that *P. carduorum* was able to infect musk thistle, cause severe disease, and overwinter under Virginia field conditions. However, because of the repeated inoculations, the development of the rust and disease severities attainable under nonmanaged conditions could not be evaluated precisely. Results did show that this pathogen poses no threat to the nontarget plants tested, since no or only minimal infection of nontarget plants was observed in the presence of large amounts of inoculum that led to severe disease of musk thistle. Rust was observed in several musk thistle populations within 10 to 12 km of the release site in the summer of 1989 (2), but populations at greater distances were not surveyed at the time.

The objectives of this study were: (i) to describe the geographic spread of *P. carduorum* in the years since its release, and (ii) to monitor the population development of *P. carduorum* under nonmanaged conditions (in contrast to the inoculated field plots) and evaluate factors that may limit population development.

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MATERIALS AND METHODS

Methods used for establishment and inoculation of field-release plots have been described previously (3). The release site was 6 km west of Blacksburg. Musk thistle rosettes were inoculated with urediniospores on 29 October 1987, several additional times in the spring and fall of 1988 and 1989, and in the spring of 1990. Disease developed from the fall inoculations and most spring inoculations, but moderate to severe disease development occurred at the release site only in June and July of each season (1988 to 1990); disease severity was light or not detectable at other times of the year (3).

Survey of spread of *P. carduorum*. Musk thistle plants occurring naturally within 12 km of the test plots were inspected repeatedly for the presence of rust from late June through August 1988, and from May through August 1989. No attempts were made to determine further spread and distribution until a survey of a wider geographic area was undertaken in June and July 1992. First, musk thistle populations were examined for the rust disease at sites within a 70-km radius from the release site, but the search was extended to greater distances as new discoveries of rust-infected musk thistle were made. Information about musk thistle occurrence was obtained from county extension agents, plant pathologists, entomologists, and weed scientists in several states. Trips to more distant locations also were based on published information about musk thistle distribution (7).

As it became clear that *P. carduorum* had spread extensively into several states, selected scientists in Alabama, Arkansas, Florida, Georgia, Iowa, Kentucky, Missouri, North Carolina, North Dakota, Ohio, and Oklahoma were asked to survey for rust on musk thistle and report both positive and negative (musk thistle examined but no rust detected) observations.

Population dynamics. Five sites with musk thistle populations were used; these were located in Montgomery and Pulaski Counties, Virginia. Site 1 was the release site and included musk thistle rosettes planted annually, along with volunteers, for a total of about 500 plants in a 20 × 40 m area. Sites 2 and 3 were at Virginia Polytechnic Institute and State University's Whitethorne-Kentland Farm, 8 km west of the release site and about 1.5 km apart. Site 2 was a small, fairly dense stand of volunteer plants (several plants per m^2 within a 15 × 20 m area) near a larger field of musk thistles transplanted for entomological research, for a total population of more than 1,000 plants. Site 3 contained about 100 thistles in clumps distributed over a 10 × 30 m area plus a few plants scattered outside that area. Site 4 was 15 km east of the release site and contained approximately 200 thistles scattered over a 60 × 20 m area, and site 5 was a creek-side pasture 20 km west of the original release site, with 300 to 400 thistles (0.5 to 1 per m^2). In addition to musk thistles, all sites contained grass and other vegetation ranging from 50 to 100 cm high in early summer. The history of site 5 is unknown, but all other sites had musk thistle populations since at least 1988, and *P. carduorum* was present in 1989 and 1990.

Thistles at site 1 were last inoculated and monitored in the spring of 1990 (3). Sites 2, 3, and 4 were inspected occasionally in 1990, but no pustule counts were made. In 1991, monitoring was started on 17 April, and disease was first detected on 3 May. On that date, 11 to 20 plants at each of sites 1, 3, and 4 were marked and subsequently inspected approximately every 10 days until late June. Individual leaves were tagged and examined during these visits. Occasional visits also were made in the fall. In 1992, the first inspection was made on 29 April, and 15 musk thistle plants per site were marked at sites 2, 3, and 4 on 13 May, and at site 5 on 9 June; plants were not marked at site 1 in 1992 because of the minimal amount of rust in 1991. Marked plants were examined for rust approximately every 10 days through mid-August. At every inspection, pustules were counted on five arbitrarily selected older (bottom) leaves, five middle leaves, and five young (upper) leaves. As older leaves became completely necrotic, pustules on them were no longer counted. After these plants had died in July and August, 15 young rosettes were marked at each site in early September; leaves and rust pustules per plant were counted monthly until early December 1992. On each monitoring date, samples of rusted leaves were collected from unmarked plants at each site and brought to the laboratory. Spores from at least four leaves (if available) were streaked out on water agar and incubated for 24 h at 20 to 25°C. Generally, at least 100 spores were counted to determine germinability. Only spores well separated from others were included in the germination counts. In addition, at least 100 spores were examined microscopically to determine the teliospore:urediniospore ratio.

To determine the latent period (time from infection until appearance of the first open rust pustule), greenhouse-grown rosettes were inoculated with urediniospores (10 mg/100 ml of water), incubated overnight in a mist chamber, and placed outdoors in a sunny location on a lawn. Plants were examined daily for the development of rust pustules. Timing of spring infection was determined using as an inoculum source dead, rusted musk thistle plants collected in August 1992 and held outdoors 2 km from the nearest known musk thistle population. The dead stalks were held upright by a metal wire cage and covered with polypropylene netting. From late March through early June 1993, greenhouse-grown musk thistle rosettes were placed outdoors under the dead stalks for periods of 5 to 10 days; the rosettes were then moved back to the greenhouse and observed for symptom development.

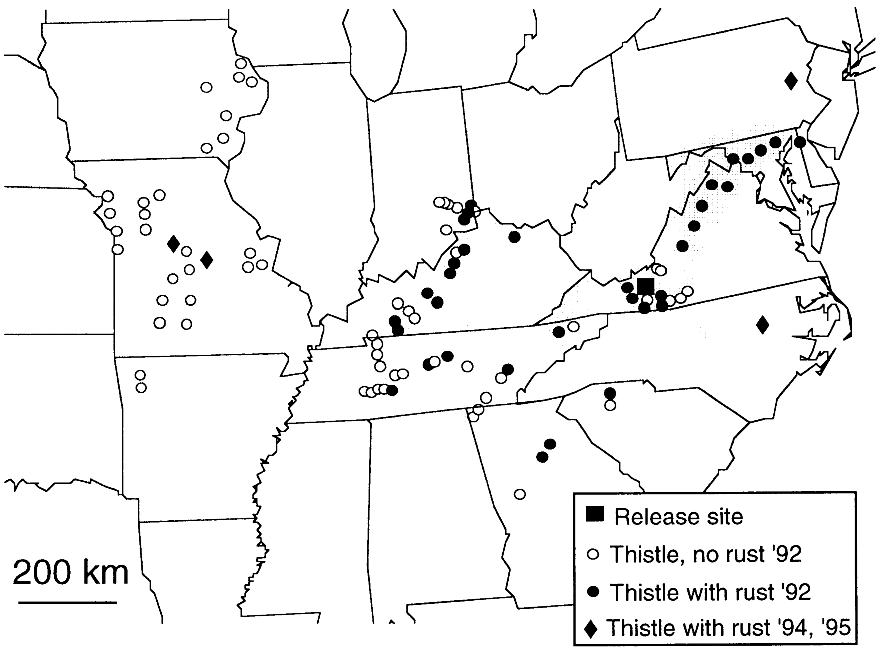

Fig. 1. Distribution of *Puccinia carduorum* in 1992, with 1994 and 1995 additions. Gray-shaded areas indicate approximate distribution of musk thistle according to Dunn (7). In southwestern Virginia, only a sample of sites visited is shown.

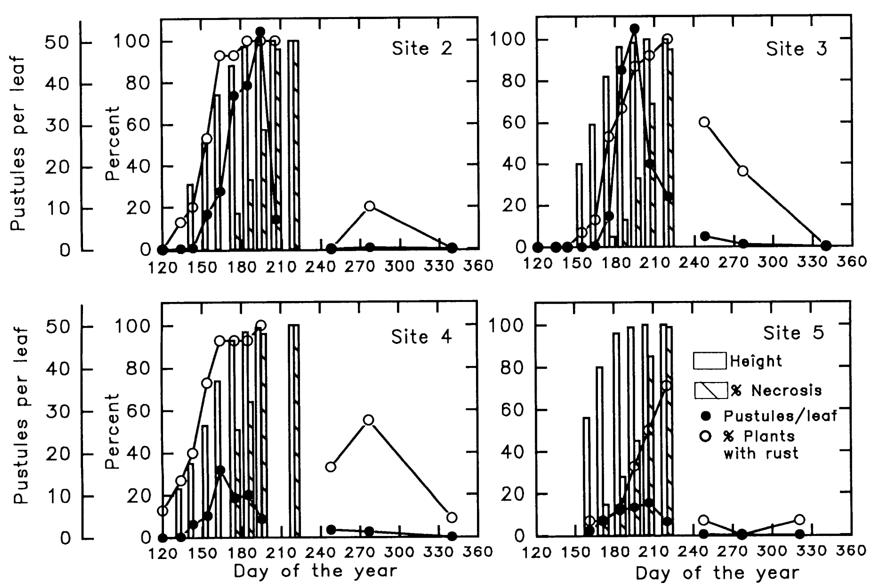

Fig. 2. Population development of *Puccinia carduorum* in relation to thistle development (plant height and percent necrotic leaf area that reflects natural plant senescence) at four sites in 1992.

RESULTS AND DISCUSSION

Spread of *P. carduorum*. In 1988, the first summer following release, rust was detected on volunteer musk thistle plants 50 to 200 m from inoculated plots in June and July. No rust was found during regular inspections at other thistle stands, 5 to 10 km from the release site. In 1989, rust was not detected at these more distant sites in late June. Between 10 and 31 July, small amounts of rust (ranging from 1 pustule on 1 plant to 1 to 50 pustules on 5 plants per site) were found at four of seven sites 5 to 11 km east and west of the release site. Pustules were well developed, some with three or four satellite pustules, indicating that infection had occurred several weeks earlier. About 50 pustules were found on one plant, indicating secondary spread. After 31 July, the majority of flowering musk thistle plants was naturally dead or dying. *P. carduorum* was observed at those four sites every year since 1989.

Survey results from 1992 are shown in Figure 1. Furthest occurrence detected was

580 km (365 miles). Reports of negative observations in 1992 (musk thistle examined but no rust found) were received from A. H. Epstein, Ames, Iowa; D. A. Johnson, Jefferson City, and B. Puttler, Columbia, Missouri; G. E. Templeton, Fayetteville, Arkansas; and R. W. Stack, Fargo, North Dakota.

In October 1994, *P. carduorum* was detected at two sites in north central Missouri (Fig. 1) by B. Puttler, University of Missouri. Through samples sent to A. Baudoin, it was confirmed that this rust was morphologically indistinguishable from the rust released in Virginia. Also in 1994, musk thistle rust was first noted in North Carolina (R. C. McDonald, *personal communication*). In 1995, a sample of *P. carduorum* collected in eastern Pennsylvania was received from C. Richwine.

Therefore, by 1992, less than 5 years after the first release, *P. carduorum* occurred throughout the major musk thistle populations east of the Mississippi River, but had probably not yet reached the concentrations in western Missouri. By 1994, rust was present in Missouri, more than 1,050 km from the release site. Although the thistle distribution data shown (Fig. 1) were from a 1974 survey (7), we believe that the main features of this distribution still apply, even though changes in local populations have occurred. For example, musk thistle has declined in western Virginia since the 1970s due to biological control by several insects (10,11). On the other hand, only one county in North Carolina was reported infested with musk thistle in 1974 (7), compared with 15 counties currently (13).

It appears likely that the current widespread occurrence of *P. carduorum* is due to the releases in Virginia beginning in 1987. This rust species had not been seen previously in the United States, except for its occurrence on *Carduus tenuiflorus* in California (18) and Oregon (8), but this isolate does not infect musk thistle (4). Other than that, it is not recorded in several comprehensive references (6,8,17), and there are no other specimens of *P. carduorum* from North America in the rust collection of the Arthur Herbarium (G. E. Shaner, *personal communication*) or the U.S. National Fungus Collection (M. E. Palm, *personal communication*). One of us (W. L. Bruckart) has observed musk thistle in Maryland since 1981 and did not see rust until well after the 1987 release. In a detailed survey of musk thistle stands in Montgomery County in the summer of 1988, we were unable to detect any rust beyond the immediate vicinity of the release site. If *P. carduorum* had been endemic at that time, it seems unlikely, based on our current experience with its population dynamics, that it would have escaped our notice. Spread over the distances reported here seems plausible in light of the well-documented dispersal capability of windborne rusts (14). Even though weather patterns in the United States often move from west to east, easterly winds are not uncommon in the mid-Atlantic states (1).

Population dynamics. Rust population development followed the same general pattern at all sites in 1991 and 1992 (Fig. 2), although the time and magnitude of population increases varied somewhat. Disease development was earliest at site 4 and latest at site 3 in both 1991 and 1992. Disease severity was low in early spring (Fig. 2) despite weather apparently suitable for infection (15). In 1991, no rust was found at sites 1, 2, and 3 in an inspection on 17 April, but on 3 May, six pustules were found in 15 min of searching at site 4, and 14 pustules were found at site 2 (20 to 30 plants per site). On 8 May, three of 11 plants selected for observation at site 3 and five of 11 plants at site 4 had a few (up to 50 on one plant) pustules. In 1992, light disease was noted on 29 April only at site 4 on three of 20 plants (1, 1, and 3 pustules per plant), and on 13 May also at site 2 on three of 15 plants (1, 1, and 12 pustules per plant), whereas no rust was found at site 3 until 2 June.

Table 1. Rates of increase of *Puccinia carduorum* pustules per leaf for periods of late spring and early summer[a]

	Start of period		Duration		
	Date	Day of year	of period	r^b	Plants
1991					
	8 May	128	6 days	0.21	8
	14 May	134	9 days	0.26	10
	23 May	143	11 days	0.17	10
	3 June	154	10 days	0.12	11
	13 June	164	11 days	0.18	14
1992					
	13 May	134	9 days	0.30	7
	22 May	143	11 days	0.24	4
	2 June	154	10 days	0.14	14
	12 June	164	11 days	0.19	18
	23 June	175	10 days	0.18	17
	3 July	185	10 days	0.16	12

[a] Data based only on plants that already had rust but no more than 10 pustules per leaf at the start of a period. Average of sites 3 and 4 for 1991, and 2, 3, and 4 in 1992.

[b] $r = (\ln y_2 - \ln y_1)/\text{time}$, where y = number of pustules at time 1 (y_1) and time 2 (y_2), and time is the period from time 1 to time 2, in days.

Rust severity increased at most sites in late May as thistle stems elongated (Fig. 2, plant height and necrosis shown to indicate stage of plant development). Peak averages of 17 pustules per leaf were reached in late June 1991 at sites 3 and 4, while no rust developed on the marked thistles at site 1 (a few pustules developed on nearby plants). Peak populations in 1992 ranged from eight to 52 pustules per leaf at the different sites, while the time of peak population densities varied from 12 June to 24 July (Fig. 2). Although rust populations were much larger in June, rates of disease increase on plants that had rust in early May were about the same in May as in June (Table 1, regression analysis of r value on date revealed slopes not significantly different from 0). The number of pustules per leaf declined following the peak, both because uppermost leaves were smaller and because greater parts of the remaining leaves became necrotic as the plants died off following seed production or were eaten by insects (only pustules on green leaf areas were counted).

Disease severity in the fall was low at all sites in 1991 and 1992, with less than three pustules per leaf (Fig. 2). There was little overall change in number of pustules between early September and early October, but by late November or early December only a very few pustules could be found. One exception was observed at site 4 from September to November 1990, when there were several late-flowering plants with hundreds of pustules each, and surrounding rosettes had more pustules than were found at other sites or in the fall of other years. The pattern of population development in natural stands generally agreed with that observed in noninoculated plots in our 1988 to 1990 field tests (site 1) (3). First rust detection in natural, noninoculated stands ranged from 29 April to 2 June. In noninoculated field plots, rust was generally not detected until the second half of May, while plants in fall-inoculated plots developed rust earlier, between late March and mid-April.

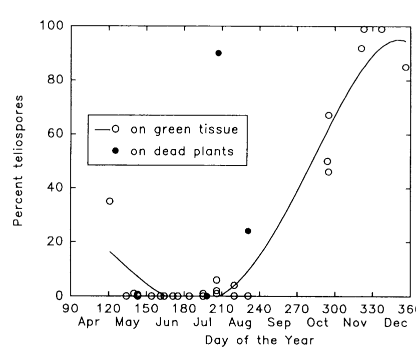

Fig. 3. Prevalence of teliospores as a percentage of all spores of *Puccinia carduorum* (urediniospores + teliospores) on thistle leaves as a function of time of the year. Curve describes the data for green leaves.

In order to investigate causes for the limited rust development in the fall and early spring, the balance between urediniospores and teliospores was examined (Fig. 3). From May to mid-July, almost all spores were urediniospores. Material collected in late July from dead thistles contained mostly teliospores in 1988 but almost exclusively urediniospores in 1989. Material collected from green plants during the late summer and fall contained an increasing proportion of teliospores but still contained many urediniospores until November. It appears unlikely that the limited rust development on rosettes in the fall is due solely to a shift to the production of nongerminating teliospores.

The germination rate of urediniospores was highly variable, ranging from 10 to 88%, regardless of whether they were collected in the summer or fall (Fig. 4). There was little evidence of any downward trend over the season; germinability of urediniospores from green leaf tissue was substantial in September and October.

Latent periods (Table 2) were slightly longer than 1 week during the summer, approximately 2 weeks in April to May and September to October, and approximately 3.5 weeks when inoculation took place in late October. Greenhouse-grown plants inoculated at later dates succumbed to frost. These data indicate that there was opportunity for several urediniospore generations during April and during September to October.

When greenhouse-grown rosettes were exposed between 23 March and 20 May 1993 to inoculum on dead musk thistle stems that had overwintered outdoors, only three orange flecks with spermogonia developed; two on a plant exposed 8 to 12 April and one on a plant exposed 12 to 20 April. The lesions later turned necrotic, and no aecia or uredinia developed on these plants. *P. carduorum* has been reported to be a brachyform rust (9), i.e., it forms no aecia, or aecia that are indistinguishable from uredinia. We observed an orange lesion with spermogonia only once in the field; they are probably easily overlooked but nevertheless appear to be infrequent.

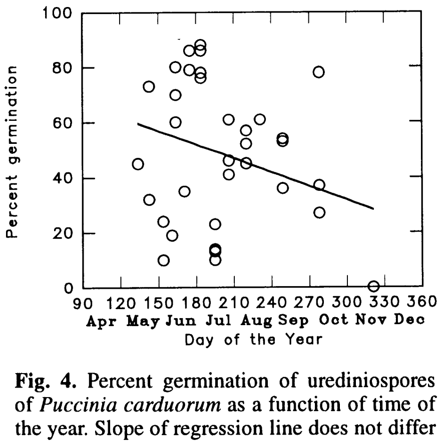

Fig. 4. Percent germination of urediniospores of *Puccinia carduorum* as a function of time of the year. Slope of regression line does not differ from 0 ($P = 0.07$).

Based on these observations, it appears that initial infections may occur in April but are infrequent in natural musk thistle stands. Disease increase in May was about the same as in June (Table 1). The apparently slow development earlier in the spring may be explained by low numbers of initial infections, cool temperatures, and longer latent periods.

Rust severity on young rosettes in the fall also was low and diminishing. Temperatures in September and October appeared to be suitable for infection, based on greenhouse data (15). Since urediniospores continued to be formed and were germinable during this period, explanations for the decline may include the fact that older rosettes are less susceptible than young ones (15), and the likelihood that spread in the rosette stage (with a majority of pustules on the abaxial leaf surfaces) may be poor, with effective increase and spread of *P. carduorum* occurring only in periods when plants have bolted and rise above the surrounding vegetation. The idea that the presence of taller, flowering plants is important is supported by one occasion where several flowering plants with many pustules were observed in September to November 1990; surrounding rosettes contained more pustules than those at other sites. An additional factor may be that spore production is probably reduced at lower temperatures.

P. carduorum appeared to be well adapted to the conditions in Virginia, and it caused at least some disease during bloom at all sites studied in both years. The fact that rust severities on young rosettes in the fall were usually low may favor the pathogen since it limits damage to its host, but it is disappointing from a biological control perspective, since young seedlings are actually the most susceptible stage (15), and severe fall infection might have led to reduced overwintering of thistles.

Table 2. Latent periods (days from infection until first open pustule) of *Puccinia carduorum* at different times of the year

Inoculation date	Latent period (days)
28 April 1990	13
14 May 1992	13
22 May 1992	14
10 June 1992	10
12 June 1990	10
25 June 1990	8
25 June 1992	8
15 September 1992	9
29 September 1988	18–28[a]
8 October 1992	17
29 October 1987	22–34[a]
30 October 1989	21–27[a]

[a] Field inoculation, not followed by daily inspections. On the first day indicated, there were either no symptoms (18 days) or yellow flecks (21 and 22 days); on the second day indicated, there were abundant open pustules.

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