Infection of Musk Thistle by *Puccinia carduorum* Influenced by Conditions of Dew and Plant Age

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ABSTRACT

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Urediniospores of *Puccinia carduorum* infected musk thistle (*Carduus nutans*) over a wide range of dew periods and temperatures and caused significant stress to rosettes. Infection resulted after 4 hr of dew at 14 C and after 8 hr of dew at 8 C, but optimal conditions of dew period and temperature were 12 hr between 17 and 24 C. Rosettes up to 10 wk old when first inoculated were very susceptible to infection; rosettes 2 wk old were most susceptible, and susceptibility declined with plant age according to the quadratic estimation $Y = 7.95 - 1.27\ X + 0.06\ X^2$ (adj. $r^2 = 0.60$). Inoculation of individual 4-wk-old plants once per week for 4 wk resulted in a reduction of rosette and root dry weights of 64 and 74%, respectively, compared with the controls. The conditions of dew and temperature needed for infection of musk thistle by *P. carduorum* are prevalent in much of North America.

Additional key words: biological weed control, rust disease, rust fungi, Uredinales

Carduus nutans L. subsp. *leiophyllus* (Petrovic) Stoj. & Stef. (sensu Moore & Frankton [6]) (musk or nodding thistle [Compositae]) is an aggressive weed of range and pasturelands in the United States (2). An active biological control program for this weed, using *Rhinocyllus conicus* Froelich, a seed-head weevil, has been established in various parts of the United States (4,13). Additional biological control agents are needed for adequate control, because some musk thistle plants flower out of sequence with the weevil's life cycle and musk thistle density will increase if a pasture is not properly managed (4,13). Recently, the possibility was presented for biological control of musk thistle with *Puccinia carduorum* Jacky from Turkey (9,10). Three isolates of this pathogen were highly aggressive on several collections of musk thistle from the United States and Canada.

Because little is known about environmental requirements for the development of *P. carduorum* in nature or the stress it can cause to *C. nutans*, experiments were designed to determine dew period and temperature necessary for infection, the effect of plant age on amount of disease, and plant weight loss that occurs as a result of infection. These experiments were part of an evaluation of *P. carduorum* for biological control of musk thistle in North America.

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MATERIALS AND METHODS

P. carduorum isolate III, collected in Turkey by R. G. Emge in 1978, was used for these experiments. This isolate was selected because it was similar to the other isolates in aggressiveness on musk thistle and in host range (10). Large-flowered *Carduus* species not susceptible to this and the other isolates (10) differed either taxonomically or were hybrids between *C. nutans* subsp. *leiophyllus* and other large-flowered *Carduus* spp. (M. K. McCarty, *personal communication*).

All inoculations were made in a turntable settling tower (5) using 1 mg urediniospores for eight plants. Two weeks after inoculation, the pustules that developed were counted in each of three disks from infected leaves. The disks, 14 mm in diameter, were outlined with a cork borer. A standard arrangement of disks was used on each infected leaf; one disk was located 3 cm and the other two were 5 cm from the apex of the leaf.

To determine the effect of plant age, *C. nutans* seeds were sown weekly for 10 wk, providing a range of plant ages. Germination of musk thistle seeds involved treatment overnight with gibberelic acid (GA_3, 10^{-3}M) before planting. Seeds were grown in a sterilized greenhouse soil mix (2:1:1, soil, sand, peat moss, v/v). Musk thistle plants up to 4 wk old (about 3 wk after emergence from the soil) were grown in 10-cm-diameter clay pots, and plants 5–10 wk old were grown in 19-cm-diameter plastic pots. Eight plants from each age were inoculated with 1 mg of urediniospores of *P. carduorum* and subsequently placed in dew chambers at 20 C for 16 hr. Two weeks later, pustules were counted.

It was necessary under our conditions to induce bolting of musk thistle to study its susceptibility at other growth stages. Six 7-mo-old musk thistle plants were grown in 25-cm-diameter plastic pots and sprayed with GA_3 while six other plants served as unsprayed controls. During anthesis, the six plants that bolted were inoculated with a suspension of urediniospores of *P. carduorum* (1 mg/20 ml of water plus Tween 20) and subjected to dew.

To determine the optimum conditions of temperature and dew for infection, inoculated musk thistle plants were subjected to 4, 8, 12, and 16 hr of dew at temperatures ranging from 8 to 36 C. Dew period is simply the amount of time plants were inside dew chambers. Three plants were used per treatment combination, and the experiment was performed twice.

Stress from infection by *P. carduorum* was determined by inoculating rosettes one, two, three, or four times on a weekly basis. Rosettes were 4 wk old when the experiment was begun. Plants to be inoculated once were inoculated the first week and allowed to grow until the experiment was terminated; plants to be inoculated twice were inoculated the first and second weeks and allowed to grow until the experiment was terminated. The other treatments followed a similar procedure. Controls were not inoculated or subjected to dew.

Two weeks after the fourth inoculation, all plants were removed from pots and soil was washed from the roots. Roots were removed from tops (rosettes), and fresh weights were recorded for individual plant parts. Plant parts were dried overnight at 105 C, and dry weights were recorded. This experiment was conducted twice.

Data from these experiments were tested by analysis of variance using the

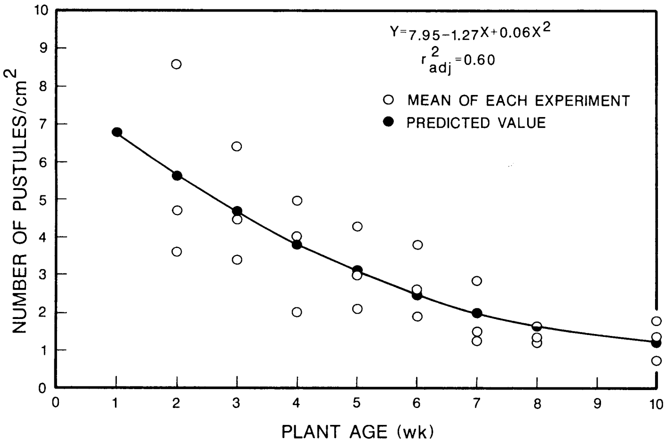

Fig. 1. Average number of pustules of *Puccinia carduorum* per square centimeter produced on rosette leaves of musk thistle 2–10 wk old at inoculation. Plants were inoculated with urediniospores in a settling tower and subjected to 16 hr of dew at 20 C. Pustules were counted 2 wk after inoculation.

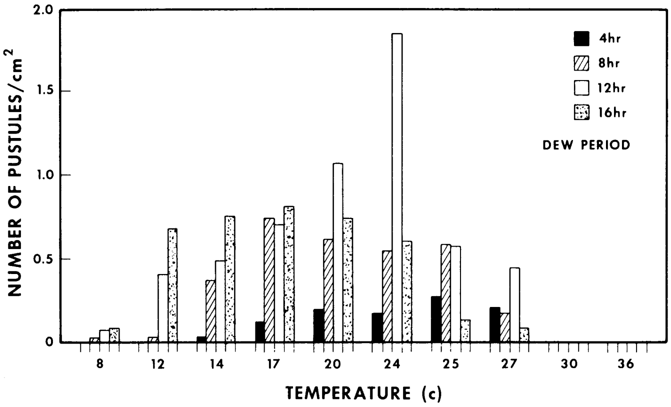

Fig. 2. Average number of pustules of *Puccinia carduorum* produced on leaves of 4-wk-old musk thistle rosettes at each of four dew periods and eight dew chamber temperatures. Plants were inoculated with urediniospores in a settling tower and subjected to dew periods and temperatures indicated. Pustules were counted 2 wk after inoculation.

general linear models procedure. Treatment data were separated with Tukey's HSD (11) in the dew period study, because temperature was analyzed by temperature classes and predictive mathematical models were not desired. Orthogonal contrasts (11) were used to determine treatment effects in the stress study. Regression analysis (11) was used to describe effects of plant age on amount of infection.

RESULTS

Musk thistle was very susceptible to *P. carduorum* up to 10 wk after planting (about 9 wk after emergence from the soil). Plants of *C. nutans* inoculated 2 wk after planting were the most susceptible, and susceptibility of the rosettes to *P. carduorum* declined with age (Fig. 1) according to the quadratic equation $Y = 7.95 - 1.27\,X + 0.06\,X^2$ (adj. $r^2 = 0.60$).

When 7-mo-old musk thistle rosettes were forced to bolt under greenhouse conditions, the plants developed pustules 2 wk after inoculation on stems, cauline leaves, pedicels, and bracts as well as on rosette leaves. Rust pustules continued to enlarge and produce viable urediniospores until the plants became senescent. However, the number of pustules per unit area was much less than that observed on younger rosettes.

Infection of musk thistle by *P. carduorum* occurred from 8 to 27 C (Fig. 2). Infection by *P. carduorum* also occurred at 4–16 hr of dew, although the amount of infection was limited with only 4 hr of dew. Mean separation with Tukey's HSD indicated the optimal dew period to be 12 hr regardless of temperature, and the optimal temperature range was 17–24 C regardless of dew period.

Weight of rosette and root tissue was significantly less than that of the controls as a result of inoculating musk thistle with *P. carduorum* (Fig. 3). Dry weights of rosettes and roots were reduced an average of 55 and 57%, respectively, compared with respective control values of 2.71 and 1.80 g. One inoculation did not always reduce the fresh or dry weight of plant parts by a statistically significant margin from the controls, but two or more inoculations resulted in a significant reduction from the controls ($P < 0.01$). Differences in fresh or dry weight from two, three, or four inoculations also were not statistically significant on the basis of orthogonal contrasts. When rosettes were inoculated weekly for 4 wk, dry weight was reduced 64 and 74%, respectively, for rosettes and roots compared with controls.

DISCUSSION

On the basis of results of the dew period/temperature studies, *C. nutans* is susceptible to *P. carduorum* over a wide temperature range. Combinations of dew period and temperature suitable for infection, as revealed in this study, are common in North America, where musk thistle is a problem, especially in late summer and early spring when musk thistle is in the seedling and rosette stages.

Although musk thistle is very susceptible to infection by *P. carduorum*, it is not likely to be killed outright by the pathogen. Rather, plants will probably be stressed so that they are less able to compete with other plants, or they may be more susceptible to cold and drought injury. Although plants became less susceptible with age and repeated inoculations caused a significant weight reduction in this study, plants were not killed.

Similar effects were reported by Emge et al (3) and Adams and Line (1) with regard to infection of skeletonweed (*Chondrilla juncea* L.) by *P. chondrillina* Bubak & Sydow, a pathogen that appears similar to *P. carduorum* in aggressiveness and infection requirements. Emge et al (3) found that skeletonweed plants with rust produced 94% fewer seeds that were lighter and less vigorous than controls. In a 3-yr field study in the state of Washington, Adams and Line (1) found

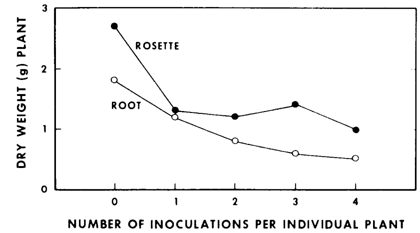

Fig. 3. Average dry weight of roots and rosettes of individual musk thistle rosettes inoculated up to four times on a weekly basis with urediniospores of *Puccinia carduorum*. Weights were taken for all treatments 2 wk after the last (fourth) inoculation.

that *P. chondrillina* reduced numbers of flowers, seed viability, and plant size each year. Although population of skeletonweed was not reduced, plant populations were "stable" for the duration of their experiment (1). It will probably be several years before populations of skeletonweed decline in undisturbed settings where they occur in the Northwest, particularly since *C. juncea* is a perennial.

Olivieri (7) reported that repeated inoculations of *C. pycnocephalus* L. with *P. cardui-pycnocephali* Syd. under greenhouse conditions reduced plant growth, reduced numbers of florets produced, and increased the time to flowering compared with uninoculated controls. *P. cardui-pycnocephali* and *P. carduorum* are very similar and may be the same species (12).

Little information is available regarding the effects of rust infections on root growth of plants. However, early-season release of the yellow nutsedge rust pathogen (*P. canaliculata* (Schw.) Lagerh.) caused a significant reduction in tuber set of diseased plants (8). A significant effect of infection on root weight occurred with *P. carduorum* in our study, but more detailed study is necessary before the significance of this aspect of plant disease on survival of musk thistle can be assessed properly.

It is reasonable to assume that the pathogen could become established and proliferate on musk thistle in North America. It also seems likely that the uredinial stage of the pathogen will add considerable stress to musk thistle without interfering with the activity of insect species introduced for biological control of *C. nutans*.

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