Biology of *Puccinia chondrillina* in Washington

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ABSTRACT

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Puccinia chondrillina is an autoecious, macrocyclic rust fungus, but in Europe where it is indigenous, pycnia and aecia are rare. In Washington, where *P. chondrillina* was released in 1978 for control of rush skeletonweed (*Chondrilla juncea*), all stages of the life cycle of the pathogen were consistently found. Uredia and telia were observed each year subsequent to release of urediospores in weed-infested areas, and pycnia and aecia were observed in the spring of 1980 and 1981. All stages of *P. chondrillina* were also produced in the greenhouse. Teliospores collected from field sites germinated and produced basidiospores that infected leaves and stems of *C. juncea*, but teliospores produced in the greenhouse did not germinate. Pycnia appeared 16 days after basidiospore-producing teliospores were placed over the plants, and uredinoid aecia appeared in a circle around the pycnia 14 days after cross-fertilization. Several uredial generations were observed. Teliospores developed in stem lesions on plants in the field beginning in July and were capable of germinating in November. Pycnia and aecia, however, were not observed in the field until early spring. During the unusually mild winter of 1980–1981, the rust also overwintered as sporulating uredia and latent uredia and pycnia.

Additional key words: biological control, weeds.

Puccinia chondrillina Bubak & Syd. is an autoecious, macrocyclic rust fungus parasitic on rush skeletonweed, *Chondrilla juncea* L. (Compositae). This pathogen is native on rush skeletonweed in Europe and has been introduced into Washington to control the weed. In Mediterranean Europe, teliospores of the pathogen are produced late in the growing season at the onset of flowering of the host, but according to Hasan (5), they play no part in survival of the pathogen. Arthur (1) considered the teliospore-sexual phases to be of minor importance in the United States.

In Europe, according to Hasan (6), seedlings appearing in the fall and rosettes developing from established root systems are infected in the fall by urediospores from the previous season's flower stems. The rust spreads rapidly from rosette to rosette throughout autumn, slowly during the winter, then rapidly again in the spring. When rosettes begin to bolt during late spring, developing stems may become infected and the amount of damage to the flowering stems depends on when they are infected.

According to Hasan (5), *P. chondrillina* is not seriously affected by climate as long as there is sufficient overnight humidity to promote spore germination and penetration. The rust occurs from the wettest (1,200 mm annual rainfall) to the driest (340 mm) Mediterranean regions in Europe. In Australia, where the fungus was also introduced, it occurs in regions ranging from dry with predominantly winter rainfall in the west, through an area of increasing rainfall, to an area with predominantly summer rainfall in the north (3).

Hasan and Jenkins (7) reported that the latent period (from inoculation until the first sporulation) was 6 days at 30 C, 8 days at 25 and 20 C, 20 days at 15 C, 28 days at 10 C, and 43 days at 5 C. Emge et al (4) reported a similar latent period (7–12 days at 18–24 C) for isolate PC-16 of *P. chondrillina* on a California biotype of skeletonweed. Blanchette and Lee (2) observed that the time from inoculation to appearance of chlorotic flecks was 21, 10, and 7 days at 8, 16, and 24 C, respectively.

Emge et al (4) screened two Washington biotypes and a southern Idaho biotype of the weed, using seven isolates of *P. chondrillina* obtained from various parts of the world. The early-flowering biotype from Washington was resistant to all seven isolates. The late-flowering biotype from Washington was susceptible to four isolates (isolate PC-16 from Eboli, Italy, and three isolates from Maryland and Virginia). Only isolate PC-1 from Eboli, Italy, attacked the southern Idaho biotype.

Isolate PC-16 was released in Spokane and Whitman counties in eastern Washington during the fall of 1978 and the spring of 1979. Before its release, the pathogen's ability to survive in Washington, its life cycle in Washington, the relationship of the spore stages to the stages of plant growth, and the potential effect of the rust on the late-flowering biotype of rush skeletonweed were unknown. This

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study is one of two on the potential of *P. chondrillina* as an agent for biological control of rush skeletonweed in Washington. This study includes experiments and observations on the life cycle and epidemiology of the pathogen conducted in the greenhouse under controlled conditions and in the field under natural environmental conditions.

MATERIALS AND METHODS

Rush skeletonweed seed for greenhouse studies was collected from several sites in Spokane County and stored at room temperature in cardboard containers until used. All plants were grown in a nonsterilized potting mixture consisting of 20 L of peat, 16 L of sand, 4 L of Palouse silt loam, 20 L of vermiculite, 12 L of perlite, 115 g of lime, and 225 g of 14-14-14 Osmocote fertilizer (Sierra Chemical Co., Milpitas, CA 95035). Seedlings were started by sowing the seeds on top of the potting mixture and covering them with 0.5 cm of vermiculite. Seedlings were transplanted to individual pots when they had four to six leaves. Before inoculation, all plants were grown in a greenhouse at 5–15 C under natural light supplemented with fluorescent light to provide a 16-hr photoperiod.

In 1978, urediospores of isolate PC-16 of *P. chondrillina* obtained from R. G. Emge, USDA, Plant Quarantine Laboratory, Frederick, MD, were disseminated in rush skeletonweed-infested areas near Nine Mile Falls (site 1) and in Spokane (site 2) by personnel of the Spokane County Weed Board. In 1979, urediospores collected from plants at the release sites were used to inoculate the late-flowering biotype grown in the greenhouse. Urediospores produced on infected plants were collected with a vacuum spore collector, air-dried, and stored at 5 C in small, screw-capped vials for use in subsequent experiments.

For field and greenhouse studies that did not require precise quantitative control of inoculum, plants were inoculated with urediospores in talc by means of a DeVilbiss hand duster. After inoculation in the greenhouse, plants were placed over water at 23 C in a dew chamber inside a cooler at 5 C. This provided moisture for germination of the spores. After 12–16 hr, inoculated plants were placed in the greenhouse to allow development and sporulation of the pathogen. In field studies, urediospores were generally released in the evening to take advantage of expected dew periods; successful germination and infection depended on the natural environment.

Rust samples were periodically collected from field sites and examined by light microscopy to determine the stages of the rust on the infected plant parts. Urediospores and teliospores collected throughout the year were tested to determine the viability and survival characteristics of each spore type in Washington. During the winter of 1980–1981, infected rosettes from near Nine Mile Falls were transplanted to the greenhouse to determine if rust mycelia overwintered within the plant.

Old stems containing teliospores were periodically collected from the field and placed on a rack above uninfected rosettes in metal towers (15 × 15 × 45 cm). The infected stems were thoroughly drenched by a fine spray of distilled water each day, and the towers were covered with a glass plate to maintain moisture on the plants. The towers were kept at a diurnal temperature of 6–20 C. New sets of rosettes were placed under the teliospores on alternate days. After exposure, the plants were incubated at a diurnal temperature of 6–20 C for 3 wk and observed for symptoms. If pycnia developed, the plants were cross-fertilized by touching numerous pycnia at random with a fine-bristle brush wetted with distilled water. The plants were then observed for 3 wk for formation of aecia.

Teliospores that developed on plants in the greenhouse were treated by the same method to determine the ability of the spores to germinate and infect. In addition, greenhouse-produced teliospores were treated before inoculation to stimulate germination. Stems with teliospores were placed (i) at −5 C for 1, 2, 3, and 4 wk; (ii) at −5 C for 7 days, then at 5 C for 7 days, sampled, and the process repeated, ie, samples were made at 1, 3, and 5 wk; or (iii) at −5 C for 6 days, wetted continuously under running water for 7 days, then wetted 2 days over plants, dried 2 days, and the process repeated for a 2-wk period, according to the method of Johnson (8).

To measure latent period (the time from inoculation until 50% of the pustules sporulated), bolting rush skeletonweed plants that still had rosette leaves were inoculated with urediospores in the greenhouse, placed in a dew chamber for 12 hr, then placed at different diurnal temperature regimes (Table 1). The latent periods for rosette leaves, stem leaves, and stems were determined by counting the number of pustules each day from the appearance of the first pustule until the total number on a leaf remained constant for 2 days.

RESULTS

Uredia were observed on rosette leaves in the spring of 1979 after urediospores were released in the fall of 1978. After the rosette leaves were shed, no uredia were observed during July, August, or September at any release site. Uredia were again found in October on stems and stem leaves, and teliospores were first noted on stems in November 1979 (Fig. 1).

Germination of teliospores collected from the field during February 1980 near Nine Mile Falls was observed in the greenhouse. Basidiospores germinated and infected the leaves and stems of *C. juncea*, and pycnia formed 16 days after inoculation. The pycnia appeared as chlorotic lesions with a raised center and a small drop of exudate containing pycniospores (Fig. 2A). Fourteen days after cross-fertilization of the pycnia, aecia encircled fertilized pycnia (Fig. 2B). In a subsequent greenhouse study, aeciospores

TABLE 1. Effect of temperature on the latent period of *Puccinia chondrillina* on the late-flowering rush skeletonweed in Washington

Temperature (C)			Latent period (days)[z]		
Low	High	Mean	Rosette leaf	Stem leaf	Stem
10	14	12	11 a	...	...
9	23	16	13 b	12 a	12 a
7	31	19	14 c	13 b	12 a
6	21	14	16 d	...	...
3	10	6	17 e	...	...

[z] Mean value of nine observations. Numbers in the same column with the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

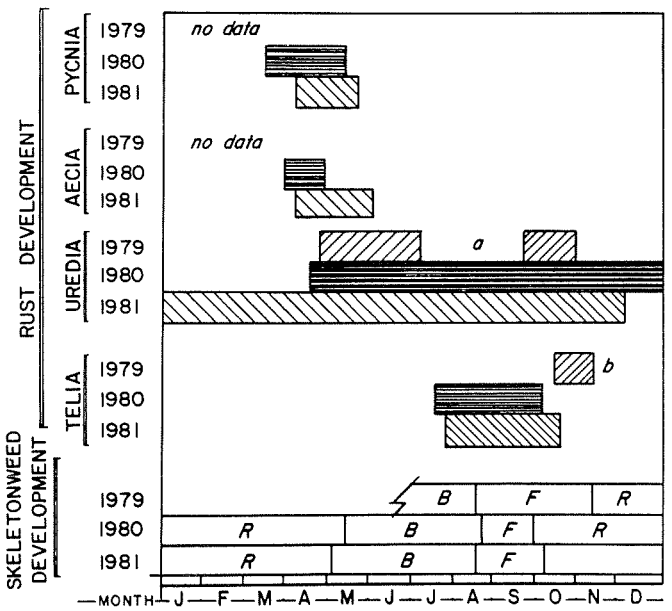

Fig. 1. Relationship of the stages of rush skeletonweed development to the stages of *Puccinia chondrillina*. The plants overwinter as rosettes (R), bolt from May through August (B), then flower (F). **A,** No uredia were observed during July, August, or September 1979. **B,** Telia were present throughout the winter on dead flower stems but not on new fall growth.

and urediospores (Fig. 2C) appeared 11–16 days after inoculation, depending on temperature, and teliospores formed in old uredia on leaves or stems (Fig. 2D).

During 1979–1980, uredia were observed in the field as late as November but not during December, January, or February (Fig. 1). Pycnia were first observed in March and aecia were observed in April. During April and May, uredia increased on rosettes of *C. juncea* in isolated patches. As the plants began to bolt, uredia developed on the stems and stem leaves. The rust continued to spread throughout the weed population, but the most severe infections were still localized. In July, teliospores began forming in older stem lesions at the base of the plant and uredia continued to spread higher on the plant.

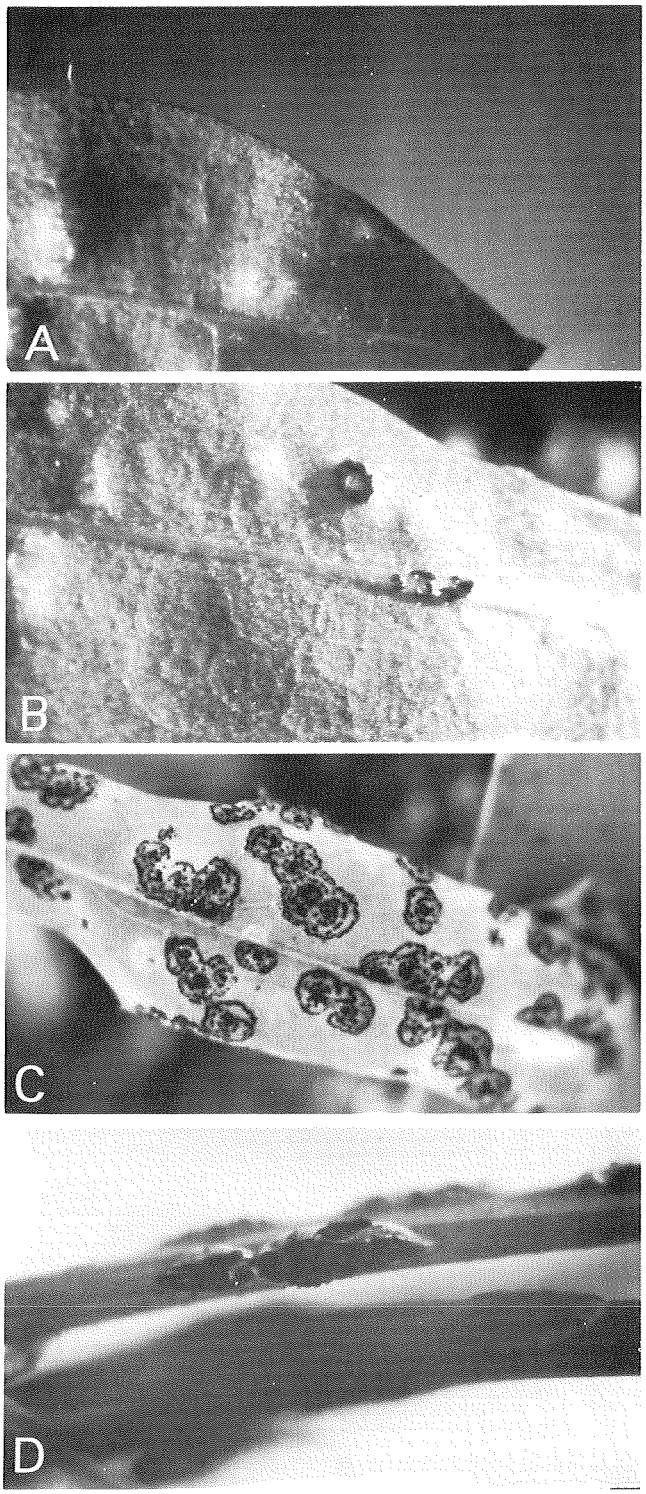

Fig. 2. Stages of *Puccinia chondrillina* on skeletonweed. **A,** Pycnia on a rosette leaf, **B,** aecia surrounding a fertilized pycnium, **C,** uredia on a leaf, and **D,** uredia and telia on stems.

New rosettes began to emerge from old root systems late in September, but the flower stems were killed by frosts in October. Teliospores collected during November 1980 germinated in the greenhouse. Uredia increased on new rosette leaves during the fall until 100% of the plants were infected. The rust survived as uredia during the unusually mild winter of 1980–1981 (mean temperatures for December, January, and February were 4.5, 3.5, and 3.7 C, respectively, whereas mean temperatures the previous year for those three months were 1.7, −5.8, and 0.7 C). Uredia were present on or developed from latent infections on 81% of the rosettes transplanted from site 1 to the greenhouse in January 1981. Pycnia developed on 38% of the transplants. At site 2, uredia were observed on 40% and pycnia developed on 25% of the transplants. In March, uredia were observed on 77% and pycnia on 38% of the transplants from site 1. At site 2, uredia developed on 25% and pycnia on 12% of the transplants. Pycnia appeared on the transplanted plants 7–14 days after they were brought into the greenhouse. Pycnia and aecia were observed in the field from early March to June 1981. Pycnia, aecia, and overwintering uredia provided a uniform, abundant inoculum for the summer of 1981.

No infections were obtained from teliospores produced in the greenhouse, whereas infections were obtained consistently from teliospores collected from the field. Attempts to germinate the teliospores produced in the greenhouse were not successful.

The length of the latent period was inversely correlated with the minimum temperatures at night ($r = 0.931^*$) but not the daily maximum temperature. Latent period increased as the night temperature decreased. The latent period on rosette leaves ranged from 11 days at the highest (10 C) night temperature to 17 days at the lowest (3 C) night temperature (Table 1). Latent periods on stems and stem leaves were 1–2 days shorter than latent periods on rosette leaves, ranging from 12 to 14 days. The latent period on stems was significantly different from that on stem leaves only at the 7–31 C temperature range.

DISCUSSION

In Washington, *P. chondrillina* shows its complete life cycle by producing all five spore stages. This contrasts to the situation in southern Idaho and in other areas of the world. In southern Idaho, where isolate PC-1 from Eboli, Italy, is being used on a different biotype of rush skeletonweed (4), only uredia have been observed in the field or under controlled conditions in the greenhouse at the University of Idaho. Uredia, therefore, are probably the normal means of survival for *P. chondrillina* in most of its range, since pycnia and aecia have rarely been observed and since telia are not believed to be important in survival even when they occur (5). Apparently, isolates of *P. chondrillina* differ not only in the biotypes they can infect but also in life cycle and survival characteristics on the different biotypes.

The rust fungus appears to have survived the winter of 1979–1980 as teliospores, although it may have survived as mycelia in latent infections in rosette leaves. The mean temperatures in December, January, and February were near normal for Washington. In contrast, the mean temperatures for these same months during 1980–1981 were well above normal. During this unusually mild winter, the pathogen survived as sporulating uredia, teliospores, and mycelia in latent uredia and pycnia. The ability of the pathogen to overwinter as teliospores may be important during the colder winters in Washington.

The ability of isolate PC-16 to produce the sexual cycle in Washington may be useful in improving the virulence of the pathogen. The sexual cycle permits genetic recombination, which may give rise to races of the pathogen capable of attacking the early-flowering biotype or other biotypes. Such races may arise naturally or may be produced in the laboratory from crosses made with different isolates.

The growth pattern of rush skeletonweed in Washington is similar to that described for the plant in other geographic regions. It is well adapted to take advantage of the wet fall and spring and to

withstand the dry summers of Washington. Leaves are shed as the plant matures, thereby reducing the transpiring surface area of the plant to conserve water. The long taproot gives the plant access to water during dry periods. The shedding of leaves plays an important role in the epidemiology of the rust. Infected plant parts and sporulating pustules are shed in the normal growth cycle of the plant. Thus, even though the percentage of the remaining plant surface (the stem) that is infected may remain unchanged, the total number of pustules and the amount of available inoculum are dramatically less. At the same time, the amount of healthy tissue available for colonization is reduced. Inoculum potential is also reduced by the conversion of active uredia on the stem to telia during the latter part of the growing season. These factors retard the increase and spread of the rust, but the rust already established on the plants continues to cause damage. Because the rust in the plant tissue extends beyond the lesions, the actual diseased area is greater than indicated by the area covered by sporulating pustules.

When using diurnal temperature ranges to determine the latent period for the rust, we discovered the latent period was shorter than that for constant temperatures similar to the mean diurnal temperature. Rust development was correlated with (low) night temperature. Apparently, the rust does not develop or develops at a slower rate below a critical temperature. Increasing night temperatures decrease the latent period. Thus, *P. chondrillina* may be able to increase at a faster rate in the spring or fall than expected from results of using constant temperatures to determine latent period. In months when the mean temperature was below 5 C, however, infections remained latent until the weather became warmer.

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