Epidemiology of *Puccinia chondrillina*, a Rust Pathogen for the Biological Control of Rush Skeleton Weed in the United States

R. G. Emge, J. Stanley Melching, and C. H. Kingsolver

Former research plant pathologist and research plant pathologist, Agricultural Research, Science and Education Administration, USDA, Plant Disease Research Laboratory, Frederick, MD 21701, and adjunct professor of plant pathology, Department of Plant Pathology, The Pennsylvania State University, University Park 16802.

Acknowledgment and appreciation are expressed for the excellent cooperation of state university and state department of agriculture personnel in the states involved, of Australian authorities, and of state and federal regulatory agencies within the United States. We thank Dennis R. Johnson for his fine technical support. We especially acknowledge Lloyd Andres, Biological Control of Weeds Laboratory, USDA, Albany, CA, for early encouragement and sustained cooperation and support.

Mention of a trademark or proprietary product does not constitute a guaranatee or warranty of the product by the USDA and does not imply approval to the exclusion of other products that also may be suitable.

Accepted for publication 13 January 1981.

ABSTRACT

Emge, R. G., Melching, J. S., and Kingsolver, C. H. 1981. Epidemiology of *Puccinia chondrillina*, a rust pathogen for the biological control of rush skeleton weed in the United States. Phytopathology 71:839-843.

Populations of rush skeleton weed (*Chondrilla juncea*) from California, Oregon, Washington, and Idaho showed differential reactions to infection by the rust pathogen *Puccinia chondrillina*. Among seven collections of the pathogen, four different virulence patterns were expressed on the host populations. The optimal temperature range for germination of uredospores during a 4-hr incubation on water agar was 11–18 C. The optimal dew period for infection was 16 hr at 10–21 C. In a field plot in the second season following a single artificial inoculation, rusted plants produced 94% fewer seeds, produced seed with 30% less germinability, had a 24% lower 1,000-seed weight, and had 89% less biomass than rustfree plants. Virulent collections of *P. chondrillina* were increased and supplied to workers in the western United States for release in specific areas for biocontrol of *C. juncea*. Inoculations resulted in rust initiation, pathogen survival over winter and/or summer in each area, and natural spread of the pathogen to uninoculated areas. This is believed to be the first use of an exotic plant pathogen for weed control in the United States.

Additional key words: distribution, host specificity, infection requirements.

Rush skeleton weed (*Chondrilla juncea* L.), a perennial weed of Eurasian origin (and a serious introduced pest in the wheatlands of Australia), has become naturalized and is increasing in the western United States. Ranchers, farmers, and agricultural authorities in California, Oregon, Washington, and Idaho are concerned about the spread and potential threat of the weed. Rush skeleton weed is reported to thrive in semiarid conditions and in well-drained soils (13); however, in the United States, it is not confined to these conditions.

The growth habit of *C. juncea*, its taxonomy and morphologic characteristics, and its distribution in Eurasia and the United States have been well documented (3,4,8,11–16).

The weed is a strong competitor for moisture and nitrogen. In grain fields, its tough stems foul the heads of harvesting equipment, resulting in costly delays (4). Because new plants may arise from root cuttings, tillage practices such as rod weeding and disking tend to increase the levels of infestation of the weed (12).

Rapid spread of *C. juncea* throughout the wheat area of southeastern Australia reduced yields and forced the abandonment of less productive fields. The southern Idaho infestation increased from approximately 40 ha when it was discovered in 1960 (13) to 162,000 ha in 1968. Increase in the number and size of the western infestations has caused concern that the semiarid agricultural regions of the western United States may be faced with a situation similar to that which occurred in Australia (13). Rush skeleton weed would be well adapted to the wheat-fallow cultural practices (14) of the Pacific Northwest.

Although the weed can be controlled to some degree by the application of herbicides at high concentrations, the cost is prohibitive, the treatment is not very effective, and the impact on the environment is undesirable (2). Less than satisfactory weed suppression and the cost of chemical control, in the face of knowledge of the success Australians have achieved in controlling the weed with the rust fungus *Puccinia chondrillina* Bubak & Syd. (15), prompted the establishment of a biological control project against *C. juncea* by state and federal scientists in California. Oregon, Washington, and Idaho have since established similar projects.

The study and release of an exotic organism for biological control must follow a strict protocol set forth by the USDA Working Group on Biological Control of Weeds, the USDA Animal and Plant Health Inspection Service, and by state agriculture departments. Regulatory controls have been designed primarily for insect introduction, because of the early involvement of entomologists in biocontrol. If the requirements of the protocol are met, the organism may be cleared for study and/or release for the biological control of a particular weed.

The macrocyclic, autoecious rust fungus *P. chondrillina* (1,5) attacks *C. juncea* and materially reduces the population of the weed (7). The organism occurs on its host in its native Eurasian habitat and in the eastern United States (1), but it was not found in the weed infestations of the western United States before 1977, when it was intentionally introduced.

P. chondrillina has been recorded only from the genus *Chondrilla* (5,10). Tests conducted by Australian scientists (5) and in our laboratory indicated that 44 commercial, ornamental, and weed species in 14 families were immune to infection by *P. chondrillina*. Because lettuce is the commercial plant species most closely related to *C. juncea*, and because the pathogen was to be released first in California, where lettuce is an important crop, the pathogen was tested in our laboratory on nine lettuce cultivars of commercial importance in California (Vanguard, Calmeria, Montemar, Salinas, Calmar, Prizehead, Climax, Merit, and Empire). All cultivars tested were immune to infection by *P.*

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

0031-949X/81/08083905/$3.00/0

chondrillina.

In these studies, we sought to determine the pathogenic range and virulence pattern of selected collections of *P. chondrillina* on populations of *C. juncea* from different geographic areas; to evaluate the effects of some primary environmental variables on disease establishment with certain pathogen-host combinations; and, based on results from the foregoing studies, to select and supply appropriate inocula and epidemiologic guidelines for biocontrol of skeleton weed in the western United States.

MATERIALS AND METHODS

Plant preparation. Test plants were started from seed sown in clay pots 10 cm in diameter containing a mixture of field loam soil, sand, peat, and vermiculite (2:1:1:1, v/v). When the rosettes had grown to about 3 cm in diameter, they were selected for vigor and uniformity and transplanted to provide one seedling per 10-cm clay pot.

Inoculation and incubation. Test plants were inoculated when the rosettes had six to eight leaves each about 7–8 cm long. All inoculations were made in a turntable settling tower (9). Viability of the uredospores was assessed on water agar just before inoculation, and the mass of spores released into the settling tower was adjusted to provide the desired quantity of germinable spores (spore viabilities ranged from 80–95%).

After inoculation, the plants were placed in dew chambers at controlled temperatures, and the leaves were sprayed immediately with a fine, uniform mist of distilled water. After the desired dew period in the closed, dark dew chambers, the plants were moved to a greenhouse; the greenhouse air temperature (dry bulb) was 21–25 C unless otherwise stated.

Spore germination and germ tube growth. Uredospores were seeded onto the surface of preequilibrated water agar (1.25% Difco Bacto) in plastic petri dishes on a controlled-temperature gradient plate. Immediately after seeding, the dishes were covered with lids that excluded light. Temperatures were monitored at the agar surfaces and were held within ±0.25 C of the stated "constant" temperature during the experimental periods.

A uredospore was considered germinated if it produced a germ tube as long as or longer than the minor diameter of the spore. Two hundred or more spores were counted for each determination; if no germination was found, the entire plate was scanned (3,000 or more spores) to verify that no spores had germinated. Estimates of germ tube length were based on microscopic measurements of 25 germ tubes for each determination.

Inoculum. Uredospores on dried, rusted leaves collected from several geographic areas were used to establish uredia on selected suscepts in the containment greenhouse of our laboratory. Uredospores of each collection were increased in isolation. Inocula used in all studies were uredospores either freshly harvested or taken from liquid nitrogen storage. Spores from storage were heat-shocked at 40 C for 5 min before use.

Disease evaluation. Inoculated plants were evaluated for differences in pustule appearance and pustule numbers 4 days after sporulation began. Infection density was determined from counts of three 1-cm^2 areas on each leaf of the test plants.

RESULTS

Physiologic specialization. Seven different collections of *P. chondrillina* were tested against all available U.S. host populations and a narrow-leaf (NL) population from Australia (Table 1). The populations of *C. juncea* were morphologically similar, except that Aust.-NL (from Australia) and N. Idaho had narrower leaves than the others. Groups of eight 5-wk-old rosettes of *C. juncea* of each population were inoculated with 4 mg of viable uredospores of each of the seven collections of the pathogen. A dew period of 20 hr at 19.5 ± 0.5 C immediately followed inoculation.

Sporulation began with the compatible pathogen-host combinations within 8 days of inoculation. No differences in pustule types were noted, but marked differences were found in the average number of pustules per leaf among some of the pathogen culture-host population combinations (Table 1). Plant populations Wash.-E and N. Idaho were immune to all collections of the pathogen; Aust.-NL was immune to all but the culture from Australia; and S. Idaho was immune to all but one collection (PC-1) from Italy. All other plant populations were susceptible to four or more of the seven collections. Pathogen collections from Italy (PC-16), Maryland (PC-49), and Virginia (PC-52 and W-51) had the broadest pathogenic range, attacking three of the seven populations.

Spore germination and germ tube growth. Agar plates were equilibrated at temperatures of 5, 8, 11, 14, 17, 18.5, 20, 23, 26, 29, 32, and 35 C. Uredospores of *P. chondrillina* (culture PC-16 from Italy) were seeded on the equilibrated agar plates and incubated in darkness for 2, 3, 4, 7, or 20 hr (±2 min).

Within 2 hr, spores had germinated at temperatures from 11 to 23 C, with about 28–62% occurring at 14–18.5 C (Table 2). Germination percentages increased sharply above 8 C to about 20 C, dropping to 0% at 26 C. After 4 hr, germination was not found at 5 C. Apparently, a constant temperature of 5 C is quite near the lower limit at which the spores of this organism can germinate on agar. At 26 C, no germination occurred even after 20 hr. The upper limit for spore germination is above 23 and below 26 C—probably closer to the upper value. At favorable temperatures for germination (ie, 11–18.5 C), incubation time beyond 4 hr resulted in little further germination. This was true to a lesser degree also at 20 C. At 8 and 23 C, little increase occurred after 7 hr.

Germ tube elongation after 4 hr of incubation was greatest from 14 to 20 C (Table 3). By 20 hr, tubes of 500 μm or longer were produced at temperatures from 8 to 18.5 C. About 1–2% of the spores produced two germ tubes at all temperatures where germination occurred. Characteristic 45–90° bends in germ tubes occurred in 20–60% of the tubes; the tendency was more pronounced at the higher temperatures. During the 20-hr incubation, 10–20% of the germ tubes branched. During 4-, 7-, and 20-hr incubation periods, a few (1–2%) of the germ tubes at 5, 8, and 11 C produced at their tips appressoriumlike, roughly circular bodies about two to three times larger in diameter than the width of the germ tube.

Infection studies. *Latent period.* During May, June, and July 1978, infectivity tests were conducted with collection PC-16 and the California population of *C. juncea.* Inoculated plants were held in a

TABLE 1. Disease ratings[a] of populations of *Chondrilla juncea* inoculated with collections of *Puccinia chondrillina*

Collections of *Puccinia chondrillina*		Populations of *Chondrilla juncea*						
Code no.	Source	Calif.	Ore.	Wash.-SW	Wash.-E	N. Idaho	S. Idaho	Aust.-NL
PC-A-NL	Australia	0	0	0	0	0	0	3
PC-48	Beltsville, MD	2	1	0	0	0	0	0
PC-16	Eboli, Italy	4[b]	4[b]	3[b]	0	0	0	0
PC-49	Dickerson, MD	4	3	4	0	0	0	0
PC-52	Fredericksburg, VA	4	2	3	0	0	0	0
PC-1	Eboli, Italy	0	1	0	0	0	4[c]	0
W-51	Woodbridge, VA	3	3	3	0	0	0	0

[a]0 = No infection; 1 = one to nine pustules per leaf; 2 = 10–24 pustules per leaf; 3 = 25–49 pustules per leaf; 4 = more than 50 pustules per leaf.
[b]Released in California, Oregon, and Washington (southwest of Spokane).
[c]Released in southern Idaho.

greenhouse at 18–24 C. The latent periods (time between inoculation and initial sporulation) ranged from 7 to 12 days, with an average of 8.5 days. Fluctuations in temperatures and light conditions among tests might account for the range of latent periods observed. In other tests with different collections of the pathogen and host populations, no consistent differences occurred in latent periods among compatible combinations.

Inoculum density and disease. The relationship between the number of uredospores deposited on the leaf surface and disease incidence was studied with collection PC-16 and the California population; this combination had been shown earlier to result in a highly susceptible reaction. Leaves 7–8 cm long were exposed to the spore shower in the settling tower; six to eight leaves per pot and six pots per inoculation were used. Fifteen inoculations were made at each level of inoculum applied (0.25, 0.5, and 1.0 mg of viable uredospores).

After inoculation, a 16-hr dew period at 20–21 C was provided; the plants were then placed in the greenhouse. Samples of leaves were examined microscopically to determine the numbers of spores deposited at each inoculum level. Pustules were counted when sporulation occurred.

A greater number of spores deposited on leaves resulted in more pustules (Table 4). The average number of spores required to produce one pustule ranged from 8.3 to 11.6. The numbers of pustules obtained with the 1-mg inoculation were convenient for counting and for making comparisons among inoculations, and reproducibility was acceptable.

Dew period temperature and duration. Plants were inoculated and placed in dew chambers for 16 hr at air temperatures of 4.4, 7.2, 10.0, 12.7, 15.5, 18.3, 21.1, 23.8, 26.6, or 29.4 ± 0.5 C. Plants were then removed to the greenhouse, where leaves dried within 15 min. Pustules were counted when they were fully developed, and the data were analyzed by a third-order polynomial regression.

The optimal dew temperature range for disease establishment was 15.5–21.1 C (Fig. 1). Pustules developed at temperatures from 7.2 to 26.6 C; 25–30% as many pustules occurred at the extremes as at the optimal temperatures. No rust developed on plants from the 4.4 and 29.4 C dew periods.

In the studies on agar (Table 2), spores did not germinate at 26 C, whereas in the infection studies, germination must have occurred on the plant leaves in the dew chamber at 26.6 C (Fig. 1). Perhaps the leaves were at a lower temperature than the air temperature in the dew chamber during the early part of the dew period, because they were not equilibrated to the experimental temperature before inoculation as were the agar plates in the germination studies, or perhaps in the presence of dew and substances supplied by the living leaves, the uredospores were able to germinate at a higher temperature.

In another study, inoculated plants were incubated in dew chambers at 7, 10, 15.5, 21, 24, or 27 ± 0.5 C for 2, 4, 6, 8, 12, 16, or 24 hr. More pustules developed on plants from all dew temperatures (except 24 C) as the dew period was extended to 12 or 16 hr (Table 5); beyond 16 hr, little or no further increase in pustule numbers occurred. A dew temperature of 15.5 C resulted in the most pustules at 6, 8, and 12 hr of dew, and 10 and 21 C were only slightly less favorable for pustule development. The least favorable temperature at which infection occurred was 24 C; even after a 24-hr dew period, only about 0.5 pustules per square centimeter were observed. At 27 C, no infection was observed at any dew period. By contrast with the warm extremes, the coldest dew temperature (7 C) was much more conducive to rust establishment, resulting in about half as many pustules per square centimeter as the most favorable temperatures at the longer dew periods.

Although no infection occurred here in dew periods shorter than 4 hr, in other tests a very few, isolated pustules developed after a 2-hr exposure of inoculated plants to dew at temperatures from 10 to 21 C.

Impact of the pathogen on its host. A field plot for the quantitative evaluation of the impact of *P. chondrillina* on rush skeleton weed was established in Frederick, MD, in late March 1978. Skeleton weed plants from the Frederick area were started in the greenhouse, allowed to develop rosettes 10–15 cm in diameter,

TABLE 2. Percentage germination of uredospores of *Puccinia chondrillina* PC-16) on 1.25% agar in darkness[a]

Temperature (C)	Hours of incubation				
	2	3	4	7	20
5	0	0	0	1.5	2.7
8	0	2.6	40.8	80.2	84.8
11	8.3	60.7	86.0	93.9	94.8
14	57.8	82.0	94.1	94.7	97.1
17	65.5	78.4	87.9	94.9	94.6
18.5	61.6	75.7	82.5	82.8	88.9
20	36.4	68.7	69.2	72.5	81.6
23	8.0	27.7	28.1	47.3	49.8
26	0	0	0	0	0
29	0	0	0	0	0
32	0	0	0	0	0
35	0	0	0	0	0

[a]Two hundred or more spores were counted for each germination determination; if no germination was found, the entire plate (3,000 or more spores) was scanned to verify that no spores had germinated.

TABLE 3. Average length (μm) of germ tubes[a] of uredospores of *Puccinia chondrillina* (PC-16) incubated on 1.25% agar in darkness

Temperature (C)	Hours of incubation				
	2	3	4	7	20
5	0	0	0	20	132
8	0	21	30	122	500
11	29	50	92	180	602
14	40	128	132	202	660
17	49	140	145	300	600
18.5	60	138	140	338	556
20	39	111	136	298	390
23	28	48	80	128	162
26	0	0	0	0	0
29	0	0	0	0	0
32	0	0	0	0	0
35	0	0	0	0	0

[a]Average length of 25 germ tubes observed at each time-temperature combination.

TABLE 4. Spore deposition[y] and pustules produced per unit area of leaf tissue of *Chondrilla juncea* inoculated with uredospores of *Puccinia chondrillina* and calculated numbers of spores required to produce one pustule

Inoculum (mg)	Spores/cm²	Pustules/cm²	Ratio (spores:pustules)
0.25	29.0 a[z]	3.5 a	8.3:1
0.5	53.3 b	4.6 b	11.6:1
1.0	76.8 c	7.6 c	10.0:1

[y]Spore counts on leaf surfaces are total numbers of spores observed.
[z]Values within columns followed by different letters are significantly different ($P \leq 0.05$).

TABLE 5. Effects of length and temperature of dew period on infection of *Chondrilla juncea* by *Puccinia chondrillina*, expressed as mean numbers of pustules per square centimeter of inoculated leaf area[a]

Dew temperature[b] (C)	Dew period (hr)						
	2	4	6	8	12	16	24
7	0	0	0.1	0.2	1.4	3.4	2.7
10	0	0	0.4	1.5	3.4	5.5	4.1
15.5	0	0.02	1.0	2.4	4.0	5.0	4.8
21	0	0	0.5	1.8	3.0	3.9	4.1
24	0	0	0	0.1	1.1	0.1	0.4
27	0	0	0	0	0	0	0

[a]Means of 144 counts of 1-cm² areas (three areas on each of eight leaves for each of six replicate plants).
[b]Air temperatures within the dew chambers were ±0.5 C of the stated constant temperature during the dew periods.

placed in a cold frame for 3 wk to harden off, and planted on 1-m centers in a 0.1-ha field. Three rows of plants on one side of the field were kept rustfree by spraying with maneb (1.5 lb of active ingredient per 100 gal of water) weekly and after each rain. The rest of the field was uniformly inoculated with uredospores of the Frederick strain of *P. chondrillina* at the rate of 2.5 g/ha. We plan to observe this plot for 5 yr with no further artificial inoculations.

During the first season, rust became established and increased. The rusted plants were smaller and formed a less dense stand than the rustfree plants. Seed set of rusted plants was reduced by 65%, seed quality (weight of 1,000 seeds) by 32%, and seed germination by 34%.

During the second season, data were taken on seed production, rate of plant growth, biomass of aboveground parts, and percentage of flowering stalks killed by the rust. The seed set of rusted plants was reduced by 94%, seed germination by 30%, seed quality by 24%, and biomass by 89% (Table 6). The average height of the rusted plants 5 wk after bolting (about June 25) was 40 cm, whereas the rustfree plants averaged 55 cm tall. This difference in height persisted throughout the season; at maturity, the rusted and rustfree plants averaged 75 and 85 cm tall, respectively. The rusted flowering stalks died progressively; toward the end of the season, approximately 65% had died prematurely.

Release of *P. chondrillina*. A cooperative effort among this laboratory; the California Department of Food and Agriculture, Pest Management and Environmental Monitoring Program; the Oregon State Department of Agriculture; Washington State University, Agriculture Extension Service; and the University of Idaho, Department of Plant and Soil Sciences resulted in the release of *P. chondrillina* in skeleton weed infestations of each state. This laboratory acquired the collections of the pathogens, screened them for specificity and for virulence to the various weed populations, supplied the inocula appropriate for the different release sites, and outlined the infection requirements. The states made the releases and kept the necessary records.

The topography and climate varied, from the semiarid regions of central California and Washington to the moist, mountainous areas of Oregon and Idaho. The methods of release also varied, from hand dusters in each location to powered dusters and water suspensions in California and aerial application by helicopter and fixed-wing aircraft in southern Idaho. All methods of release were successful, and infection was established in each location.

The pathogen survived the winter in each weed infestation. Secondary rust pustules were found up to 3.4 km from the inoculation foci within 1 yr of the inoculations.

DISCUSSION

In our studies, uredospores of *P. chondrillina* did not germinate on agar at constant temperatures of 26 C or higher. Hasan and Wapshere (7) reported germination at 36 C but not at 40 C of uredospores of *P. chondrillina* collected in France. Differences between the fungus collections may explain this discrepancy. Also, their data were for 48-hr incubation periods, while our studies were done with incubation periods of at most 20 hr. Germination data after a 48-hr incubation would be of limited value from an epidemiologic point of view; the data from our 20-hr incubation periods probably are of limited value, because leaf surfaces seldom are wet under field conditions for this length of time, except during rain. The 2- to 7-hr germination values are most useful for epidemiologic studies.

Although many other species have been tested, plants in the genus *Chondrillia* are the only known suscepts of *P. chondrillina*. The seven morphologically similar collections of the pathogen contain at least four pathogenic races. Also, although the populations of *C. juncea* from California, Oregon, and southwestern Washington are morphologically similar, their differential susceptibilities to certain collections of the pathogen indicate that they contain different genes conditioning rust resistance. The restricted host range and demonstrated virulence of the pathogen on most weed populations satisfy two of the requirements of a biocontrol agent.

Combinations of dew temperatures and durations that are favorable for spore germination by the pathogen and infection of *C. juncea* occur commonly during the growing season in areas where skeleton weed is a problem in the western United States. The organism has also been shown to oversummer and overwinter when introduced into weed infestations in the western states.

P. chondrillina causes general weakening of rush skeleton weed and gradual reduction of the weed population. The rust drastically reduced seed production, seed germinability, and plant vegetative growth. The time required to reduce the weed population to an economically acceptable level depends primarily on the amount and distribution of the initial inoculum and, of course, the environmental characteristics of the area.

Laboratory results and field observations indicate that the collections of *P. chondrillina* chosen for release should significantly reduce the rush skeleton weed populations in California, Oregon, southwestern Washington, and southern Idaho. Acquisition and screening efforts must be continued to find collections of the pathogen virulent on the populations of *C. juncea* growing in eastern Washington and northern Idaho, where some populations have thus far proved immune to available rust collections.

An integrated approach to controlling *C. juncea*, using the rust pathogen in conjunction with other natural enemies of the weed, offers the greatest probability of success. Such a program is now under way at the Biological Control of Weeds Laboratory in Albany, CA, in cooperation with the state of California. A gall midge (*Cystiphora schmidti*) and gall mite (*Aceria chondrillae*) have been released, and preliminary results have been promising (K. Brunetti, Plant Pathologist, Biological Control Services Program, California Department of Food and Agriculture, *personal communication*). The American effort owes much to the pioneering work of Australian scientists who first used these

TABLE 6. Seed production, seed germinability, seed quality, and plant biomass of rusted and rustfree plants of *Chondrilla juncea*[a]

Factor	Rusted plants	Rustfree plants	Differences (% of rustfree)
Seed production	0.044 g per plant	0.788 g per plant	94.4
Seed germination	58.5%	83.2%	29.7
Seed quality[b]	0.34 g	0.45 g	24.4
Biomass[c]	0.913 g per plant	8.51 g per plant	89.3

[a] Data are averages from 20 randomly selected plants from each area (rusted and rustfree).
[b] Weight of 1,000 seeds.
[c] Dry weight of flowering stalks.

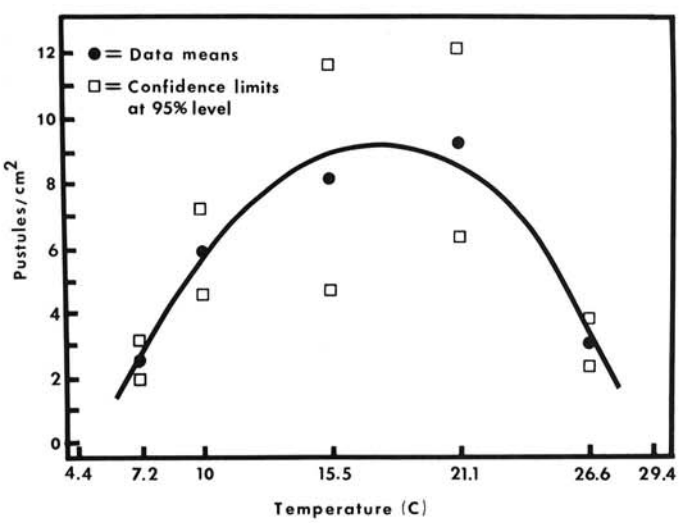

Fig. 1. The effect of selected near-constant temperatures during a 16-hr dew period on subsequent infection of *Chondrilla juncea* by *Puccinia chondrillina* as indicated by numbers of pustules.

natural enemies in their skeleton weed control program early in the 1970s.

LITERATURE CITED

1. Arthur, J. C. 1934. Manual of the Rusts in the United States and Canada. Purdue Research Foundation, Lafayette, IN. 438 pp.
2. Ehrensing, D. T., and Isaacson, D. L. 1977. Biological control of skeleton weed: Basic considerations for initiating a program in Oregon with a list of references. Oregon Dep. Agric. Weed Control Bull. No. 2. 10 pp.
3. Gaines, X. M., and Swan, D. C. 1972. Weeds of eastern Washington and adjacent areas. Camp-Na-Bor-Lee Assoc. Inc., Davenport, WA. Pages 286-287.
4. Groves, R. H., and Williams, J. D. 1975. Growth of skeletonweed (*Chondrilla juncea* L.) as affected by growth of subterranean clover (*Trifolium subterraneun* L.) and infection by *Puccinia chondrillina* Bubak & Syd. Aust. J. Agric. Res. 26:975-983.
5. Hasan, S. 1972. Specificity and host specialization of *Puccinia chondrillina*. Ann. Appl. Biol. 72:257-263.
6. Hasan, S. 1974. Recent advances in the use of plant pathogens as biological control agents of weeds. PANS 20:437-443.
7. Hasan, S., and Wapshere, A. J. 1973. The biology of *Puccinia chondrillina*, a potential biological control agent of skeleton weed. Ann. Appl. Biol. 74:325-332.
8. Hull, V. J., and Groves, R. H. 1973. Variation in *Chondrilla juncea* L. in south-eastern Australia. Aust. J. Bot. 21:113-135.
9. Melching, J. S. 1967. Improved deposition of airborne uredospores of *Puccinia graminis* and *P. striiformis* on glass slides and on wheat leaves by use of a turntable. (Abstr.) Phytopathology 57:647.
10. Morschel, J. R. 1972. Biological control agents for skeleton weed. FAO Plant Prot. Comm. S. E. Asia and Pacific Region, Q. Newsl. 15(2):23.
11. Muenscher, W. C. 1949. Weeds. The Macmillan Co., New York. 471 pp.
12. Rosenthal, R. N., Schirman, R., and Robocker, W. C. 1968. Root development of rush skeletonweed. Weed Sci. 16:213-217.
13. Schirman, R., and Robocker, W. C. 1967. Rush skeletonweed—Threat to dryland agriculture. Weeds 15:310-312.
14. Wapshere, A. J. 1970. The effect of human intervention on the distribution and abundance of *Chondrilla juncea* L. Pages 469-477 in: P. J. den Boer and G. R. Gradwell, eds. Dynamics of Numbers in Populations. Proc. Adv. Study Inst. (Oosterbeek, 1970). Cent. Agric. Publishing and Documentation, Wageningen, The Netherlands. 611 pp.
15. Wapshere, A. J. 1975. A protocol for programmes for biological control of weeds. PANS 21:295-303.
16. Wapshere, A. J., Hasan, S., Wahba, W. K., and Caresche, L. 1974. The ecology of *Chondrilla juncea* in the western Mediterranean. J. Appl. Ecol. 11:783-800.