Effects of Dew, Plant Age, and Leaf Position on the Susceptibility of Yellow Starthistle to *Puccinia jaceae*

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ABSTRACT

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Potential for biocontrol of yellow starthistle (YST) (*Centaurea solstitialis*) by *Puccinia jaceae* was evaluated in the greenhouse over a wide range of dew temperatures and periods to determine optimum conditions for infection. Disease severity (pustules per square centimeter of leaf area) was greatest when inoculated plants were exposed to dew for 12 or 16 hr at 20 C. Pustule development occurred 15 days after inoculation at 15 C and 10 days after inoculation at either 20 or 25 C. Inoculation of YST plants four times per week beginning 4 wk after planting resulted in reductions of the dry weights of root by 40% and shoots by 50% compared with the uninoculated control. Plants inoculated more than once beginning 4 wk after planting also had significantly lower root biomass than controls. YST rosettes were most susceptible 4–6 wk after planting, based on pustule counts. Nearly all leaves on 6-wk-old rosettes were uniformly susceptible to infection. Based on results of this study, *P. jaceae* has the potential to become established in and damage YST in North America.

Centaurea solstitialis L. (yellow starthistle [YST]) is a widespread, aggressive weed of semiarid rangelands and pasturelands in the western United States (8). Because of its allelopathic and poisonous properties, YST is very competitive and has low forage value in grazed pastures and rangelands. Because chemical and cultural control measures are not always economical, recently, natural enemies of YST have been considered for biological control (7,9).

The weed is of Mediterranean origin and is relatively free of effective natural pathogens in the United States (7). For these reasons, susceptibility of YST to exotic (foreign) plant pathogens from Eurasia has been evaluated. Of these, an isolate of *Puccinia jaceae* Otth., collected on YST from Turkey, is a good candidate for biocontrol (2). It is an autoecious, demicyclic rust (13) that is aggressive on YST. Infections of nontarget species are limited, so it is not likely to threaten North American agriculture or other ecosystems (2).

The objectives of these investigations were to determine environmental requirements for infection and to measure the effect of infection on YST. Dew temperature and dew period requirements for infection, latent period at different temperatures, and the effects of plant age and leaf position on susceptibility of YST to *P. jaceae* were determined. The effect of single and multiple inoculations of plants at different ages was also determined.

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MATERIALS AND METHODS

All plants were grown from seed planted in a sterile soil mix of soil, sand, vermiculite, and peat (2:1:1:1, v/v) in 12.5-cm-diameter clay pots. Seed was supplied by the California Department of Food & Agriculture from locations where YST is a major pest. Plants were inoculated between 4 and 6 wk after seeds were sown, unless otherwise stated. Plants were placed in a dew chamber at 20 C for 12 hr after inoculation unless otherwise stated. After the dew period, plants from each experiment were placed in a quarantine greenhouse at 22 C (± 2 C) with a minimum 12-hr photoperiod.

An isolate of *P. jaceae* collected by S. Rosenthal in 1984 near Ankara, Turkey, was used throughout this study. It was studied under a permit issued by the Animal and Plant Health Inspection Service (APHIS). Urediniospores were kept in a liquid-nitrogen freezer until inoculations were made. Spores were heat-shocked in warm water (40 C for 5 min) immediately after being removed from the freezer. All inoculations were made in a turntable settling tower (10) at the rate of 1 mg of urediniospores per plant. Pustules were counted 2 wk after inoculation from the three most infected leaves on each plant, unless otherwise stated. Measurement of leaf area was made with a Licor (Lincoln, NE) Lambda Model LI 3050A area meter. Disease severity was based either on the number of pustules per square centimeter of leaf area or the number of pustules per leaf.

To determine dew period and temperature requirements for infection, 1-mo-old YST plants were placed in dew chambers for either 4, 8, 12, or 16 hr immediately after inoculation. Air temperature in the dew chambers ranged from 10 to 30 C (± 2 C) in 2.5-C increments. Eight replicates were used for each treatment combination and the experiment was performed three times.

To determine the effect of temperature on latent period, YST plants were placed in a dew chamber for 16 hr at 20 C before being transferred to controlled-environment chambers at 15, 17, 20, or 25 C until pustules developed. The latent period was recorded as the average number of days until the first pustules erupted on each plant. There were six replicates in time, with five plants per replicate. Pustules per leaf were counted on the three most infected leaves of each plant in three replications in order to determine if disease severity is affected by temperature during the latent period.

The effect of plant age on susceptibility was determined by inoculating plants that ranged in age from 2 to 14 wk after planting. Eight plants in each age group were evaluated and the experiment was performed three times.

To determine the relationship between leaf position and disease severity, pustule counts were made 3 wk after inoculating 6-wk-old rosettes. Plants selected for inoculation were similar in development. After incubation, leaf position, from oldest (cotyledon) to youngest, and leaf area and number of pustules per leaf were recorded. Ten replicates were used in the experiment, which was performed three times.

Stress from infection by *P. jaceae* was determined by inoculating YST plants one, two, three, or four times per week beginning 4 wk after planting. The inoculation schedule for treatments is given in Table 1. Uninoculated plants were used as controls. Stress was evaluated in terms of shoot and root dry weights. Plants were removed from pots 3 wk after the last inoculation and soil was washed from roots. Plants were then oven-dried overnight at 105 C. Roots were severed and dry weights were recorded for individual rosette and root parts. The experiment was performed three times.

A second multiple inoculation experiment involved inoculation of plants one, two, or three times beginning 2, 4, or 6 wk after planting. The inoculation schedule for treatments in this experiment is also given in Table 1. The experiment was conducted twice with five plants per treatment in each experiment.

Data for each experiment were tested for normality with Proc Univariate and for randomness with Proc Corr for absolute values of the residuals, both from SAS (12). Data not satisfying the diagnostic tests were transformed by the natural log (ln) plus a constant; the size of the constant was adjusted until data were random and normal. The general linear models procedure of SAS (12) was used for analysis of variance, computation of regression equations, and mean separation. Means were separated with the Waller-Duncan k-ratio t test.

RESULTS

The greatest number of pustules developed on YST with 12 or more hours of dew at 20 C (Fig. 1). Data were analyzed by selected temperatures because the statistical diagnostic tests described previously indicated these were random and normal. Mean counts were not different ($P = 0.05$) at 15 or 17.5 C between the 8-, 12-, and 16-hr dew periods, but at 20 C, counts were significantly lower after the 8-hr dew period than those made after 12 or 16 hr of dew. Disease severity was very low after the 4-hr treatment.

An inverse relationship between latent period (Y) and temperature (X) was described by the following quadratic equation: $Y = 65 - 4.9X + 0.1X^2$ ($R^2 = 0.89$) (Fig. 2). The final number of pustules that developed did not differ with temperature during the latent period.

Relationship between susceptibility (Y, as pustules per square centimeter) and plant age (X) was described by the following equation: $\ln(Y + 3.5) = (-0.34 + 1.28X) - 0.17X^2 + 0.006X^3$ ($R^2 = 0.85$). The most pustules developed on plants 4 to 6 wk after planting (Fig. 3). Susceptibility of older plants decreased with age, and mean counts were 0.54 and 0.50 pustules per square centimeter on 12- and 14-wk-old plants, respectively. Leaves on 2-wk-old YST plants were not fully developed, and fewer pustules formed on them.

Dry weights of YST rosettes and roots were less ($P = 0.01$) than controls from inoculations with *P. jaceae* (Table 1). Mean dry weights of shoots and roots after four inoculations in the first experiment were 0.59 and 0.58 g, respectively, which was 50 and 40%, respectively, of dry weights in the controls.

In a related experiment, reduction in root biomass was inversely related to plant age and the number of inoculations. Root biomass of plants inoculated beginning 4 or 6 wk after planting was between 30 and 53% less ($P = 0.05$) than weights of roots in the controls, and multiple inoculations of plants beginning 4 or 6 wk after planting resulted in significantly lower root dry weights than in the controls (Table 1).

Differences in susceptibility by leaf position were not clearly evident for YST 6 wk after planting. Mean values for disease severity were highest for leaves in positions 4, 5, and 6 (Table 2), but these counts were not significantly different from those of leaves 1 through 8 ($P = 0.05$). This suggests that most leaves in 6-wk-old rosettes are similar in susceptibility.

DISCUSSION

Data from this study indicate that *P. jaceae* has the potential to infect and damage YST at temperatures and conditions of dew commonly expected in North America. Results from the dew period and temperature study are similar to those reported for other rust fungi evaluated for weed control in that optimum conditions were found to be 20 C and 12 or 16 hr of dew. Counts made after treatment at 15 and 17.5 C for 8-, 12-, and 16-hr dew periods were relatively higher in this study than those observed for *P. carduorum* Jacky on *Carduus thoermeri* Weinm. (= *C. nutans* L.) (11) or for *P. chondrillina* Bubak & Syd. on *Chondrilla juncea* L. (skeletonweed) (5). It may be important from the standpoint of epidemiology that there was no difference between an 8- and a 16-hr dew period at these temperatures. A few pustules also developed after treatment at 10 C, suggesting that *P. jaceae* can function at relatively cool temperatures, equivalent to those expected

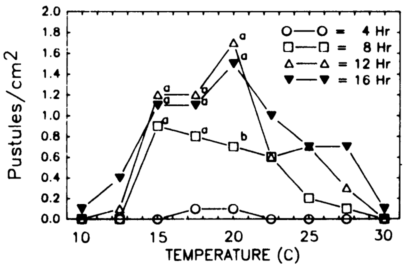

Fig. 1. Mean values for the number of pustules per square centimeter that developed on *Centaurea solstitialis* inoculated with *Puccinia jaceae* and given 4, 8, 12, and 16 hr of dew at temperatures between 10 and 30 C. Means followed by the same letter at each temperature are not significantly different ($P = 0.05$).

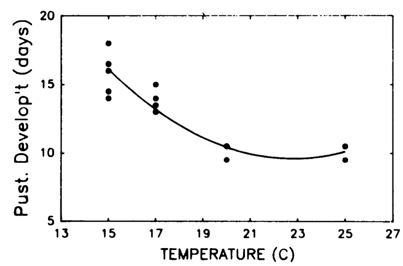

Fig. 2. Regression of time until the first pustule erupted (Y, latent period) and temperature (X) for *Centaurea solstitialis* inoculated with *Puccinia jaceae*, described by $Y = 65 - 4.9X + 0.1X^2$ ($R^2 = 0.89$).

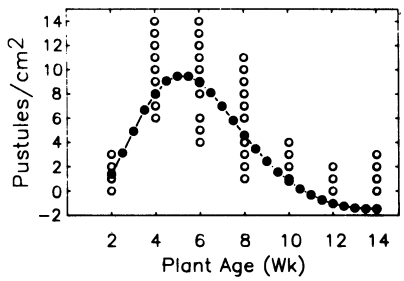

Fig. 3. Predicted median number of pustules per square centimeter (Y) developing on plants inoculated at different times after planting (X), described by $\ln(Y + 3.5) = (-0.34 + 1.28X) - 0.17X^2 + 0.006X^3$ ($R^2 = 0.85$).

Table 1. Shoot and root dry weights of *Centaurea solstitialis* inoculated up to four times with *Puccinia jaceae*

Experiment	Inoculations (no.)	Plant age (wk)[w]	Dry weight (g)[x] Shoot	Mean[y]	Root	Mean[y]
1	0[z]	···	1.20 a		0.97 a	
	1	7	0.95 b		0.80 b	
	2	6,7	0.82 b		0.67 c	
	3	5,6,7	0.65 c		0.59 c	
	4	4,5,6,7	0.59 c		0.58 c	
2	0[z]	···	6.49 ab	6.49 ab	3.19 a	3.19 a
	1	2	6.54 ab		2.57 abc	
	2	2,3	7.24 a		2.65 ab	
	3	2,3,4	7.01 ab	6.93 a	2.53 ab	2.58 a
	1	4	7.22 a		2.23 abc	
	2	4,5	7.09 ab		1.93 bc	
	3	4,5,6	6.43 ab	6.91 a	2.05 bc	2.07 b
	1	6	6.32 ab		2.04 abc	
	2	6,7	5.51 b		1.52 c	
	3	6,7,8	5.72 ab	5.88 b	1.88 bc	1.81 b

[w] Age at inoculation in weeks after planting.
[x] Means followed by the same letter in each column are not significantly different ($P = 0.05$), according to the Waller-Duncan k-ratio t test.
[y] Mean dry weights by week of first inoculation.
[z] Controls.

Table 2. Mean number of pustules per square centimeter developing at each leaf position of 6-wk-old rosettes of *Centaurea solstitialis* inoculated with *Puccinia jaceae*

Leaf number[y]	Pustules
Cotyledon	0.23 de[z]
1	0.39 abc
2	0.46 ab
3	0.40 abc
4	0.50 a
5	0.43 a
6	0.45 a
7	0.41 ab
8	0.34 abc
9	0.25 bcd
10	0.22 cd
11	0.18 de
12	0.12 ef
13	0.05 f

[y]Leaf number 1 is the oldest leaf, first to develop after the cotyledons.
[z]Means followed by the same letter are not significantly different ($P = 0.05$). Mean separation based upon data transformed by ln(pustule + 0.1).

in California where YST is a pest. The latent period of 15 days at 15 C also indicates that inoculum increase would occur, albeit slowly, at cooler temperatures.

In a similar study, Politis and Bruckart (11) reported that repeated inoculation of *C. thoermeri* with *P. carduorum* in the greenhouse caused significant reduction of root and rosette dry weights, compared with uninoculated controls. Infection in the greenhouse also occurred over a range of dew periods and temperatures commonly found in nature where musk thistle is a problem. Release of *P. carduorum* in Virginia in 1987 resulted in the establishment of the disease in the field (3).

YST is very susceptible to infection 4–6 wk after planting. Pustule count data in the plant age study (Fig. 3) and data in the multiple inoculation studies (Table 1) support this. Also, leaves are nearly uniformly susceptible 6 wk after planting (Table 2). Biomass reduction observed in the multiple inoculation studies also was greatest 4–6 wk after planting, and this may reflect the susceptibility of plants as measured by pustule counts. Although these results are encouraging, further studies that involve plants at different stages of phenotypic development are needed to show whether observations on pustule number reflect the maximum impact of the rust disease on YST or whether YST is more vulnerable to infection at later stages of development.

Successful weed management from use of a rust fungus has been reported by Cullen (4) and by Hasan and Wapshere (6) in Australia and by Supkoff et al (14) in California with *P. chondrillina* on skeletonweed. Supkoff et al (14) attributed the decline of skeletonweed populations in the field to the effects of *P. chondrillina*, rather than to a midge or a mite also released for biocontrol. More than 50% of the mortality of skeletonweed rosettes was attributed to the rust disease. Emge et al (5) found that skeletonweed with rust yielded fewer seeds and that seeds were of lower quality than controls. Reduction in the number of flowers, loss of seed viability, and reduction in plant size was reported also by Adams and Line (1) after inoculation of skeletonweed with *P. chondrillina*.

Based on our studies, we believe that *P. jaceae* is suited to environmental conditions of North America where YST occurs. The pathogen should become established without difficulty. Under favorable conditions of spread and infection expected in dense stands that occur in California, *P. jaceae* also has the potential to reduce biomass and densities of YST.

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