A Simple Quantitative Procedure for Inoculation of Safflower with Teliospores of the Rust Fungus, *Puccinia carthami*

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ABSTRACT

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A procedure is needed to determine susceptibility of safflower seedlings to teliospore inoculations of foreign accessions of *Puccinia jaceae*, a candidate agent for biological control of *Centaurea solstitialis* (yellow starthistle). The indigenous safflower rust pathogen, *P. carthami*, served as both model and basis for a future comparative study of these two rust fungi on safflower. The procedure involves inoculation of 2-day-old safflower seedlings on the hypocotyl near the base of the cotyledons with a known number of teliospores suspended in a 1- μ l drop of a 0.1% water agar and wetting agent carrier. Advantages of this procedure include (i) quantification, by spore type, of inoculum applied to each seedling, (ii) precise placement of inoculum on test plants, and (iii) conservation of inoculum. Results were verified in studies with two safflower cultivars inoculated with 0, 15, 60, or 240 teliospores of either of two isolates of *P. carthami*. Plant height and dry weight data collected 6 weeks after inoculation revealed significant interactions for cultivar $\times$ inoculum concentration and for isolate $\times$ inoculum concentration. Similar results were achieved when safflower seeds were rolled in dry teliospores of *P. carthami* but not when seedlings grew through teliospore-infested sand. The latter two approaches (dry teliospore and infested sand inoculation) require much more inoculum and are only semiquantitative. The microliter drop procedure has potential usefulness in host range and quantitative studies involving other pathogens, including bacteria, fungi, and nematodes.

Additional keywords: risk assessment

Yellow starthistle (*Centaurea solstitialis* L., YST) is a serious weed pest targeted for biological control, and *Puccinia jaceae* G. Oth is currently being evaluated for this purpose (1). Central to the risk assessment of *P. jaceae* is an host range determination that includes safflower (*Carthamus tinctorius* L.), a close relative of YST. Urediniospores of *P. jaceae* from YST or diffuse knapweed (*Centaurea diffusa* Lam.) infected safflower leaves in host range determinations (1,11), but disease was very limited and *P. jaceae* could not be maintained on currently grown safflower cultivars under optimal greenhouse conditions.

Safflower can be infected by an indigenous rust fungus, *Puccinia carthami* Corda, in the U.S. Side-by-side comparisons between *P. carthami* and *P. jaceae*

were used in the evaluation of the YST rust to clarify issues of risk concerning susceptibility of safflower to foliar infections of *P. jaceae* (3,4). These studies showed clearly that disease from urediniospores of the YST strain was much less than disease from similar concentrations of *P. carthami* urediniospores on safflower (1). For these reasons, *P. jaceae* is not considered to be potentially damaging to safflower as a foliar pathogen (1) in the event it is introduced into the U.S. for biological control of YST.

To complete the evaluation of *P. jaceae* for biological weed control, it is necessary to determine if teliospores of *P. jaceae* can initiate infection and damage of safflower seedlings. This concern is based on the potential of *P. carthami* to infect safflower seedlings, i.e., the seedling phase of the safflower rust disease (6,18) that occurs from infestations of seeds and soil with teliospores of *P. carthami* (13). Published methods for the inoculation of safflower with teliospores of *P. carthami* (12,16-19) are simple and reproducible, but they are not sufficiently precise and quantitative for the intended comparisons with *P. jaceae*. Several other methods have been described for the quantitative inoculation of plants (5, 15), but none were satisfactory for the intended research with *P. jaceae* teliospores.

The objective of this research was to develop a procedure for the quantitative inoculation of safflower seedlings with telio-

spores, using *P. carthami* as a model. Results of this research will relate directly to comparative studies between two *Puccinia* spp. (3,4) as part of a risk assessment of *P. jaceae* for biological control of YST.

MATERIALS AND METHODS

Fungal isolates, plant cultivars, and growing conditions. All experiments were conducted in 10-cm-diameter clay pots with a pasteurized greenhouse soil mix containing 80 parts Premier ProMix BX, 40 parts each of field soil, peat moss, and vermiculite, 30 parts perlite, 20 parts sand, plus lime, 10-10-10 liquid fertilizer, and AquaGro 2000-G, a wetting agent.

Two field isolates of *P. carthami*, CAL from Davis, CA, and MEX from Ciudad Obregon, Mexico, were included in these studies. The proportions of teliospores, based on microscopic observations and counts, were $82.3 \pm 4.5\%$ and $90.2 \pm 5.3\%$ for the CAL and MEX isolates, respectively. Germination of teliospores of each isolate was at least 75%. Urediniospores in these lots did not germinate due to the prolonged period of storage under refrigeration.

The safflower cultivar Pacific-1 (Pac-1; from J. Klisiewicz) was used in every experiment because it was very susceptible to *P. carthami* in earlier studies with urediniospores (1). In most of the experiments, a second cultivar, either Cal/West-74 (C/W-74; from CalWest Seeds, Woodland, CA) or UC-41 (from J. Klisiewicz), was included for comparison, because these were less susceptible to *P. carthami* in the earlier studies with urediniospores (1).

Safflower seeds were surface sterilized in 1% NaOCl plus 3 drops of polyoxyethylene sorbitan monolaurate (Tween 20)/100 ml for 5 min prior to each experiment. Seeds or seedlings were planted by placing them on the surface of the soil mix described and covering them with 2.5 cm of sand. All pots were placed in a climate-controlled greenhouse for 3 weeks at 12 to 14°C night and 15 to 20°C daytime temperatures. Pots were watered by mist twice per week with 2.5 cm of water. Plants were moved to a 21 to 25°C greenhouse for an additional 2 weeks and watered only when visible wilting of control plants occurred, unless otherwise noted. All experiments were conducted in a greenhouse facility designed for the containment of foreign plant pathogens (10).

Plant inoculations. Three methods of inoculation were evaluated: (i) dry spore,

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(ii) infested sand, and (iii) wet spore. Dry spore inoculations were made by rolling dry safflower seeds in a known weight of spores and planting as described. The final weight of spores applied was determined in each experiment by weighing the inoculum remaining after treatment. Data on spore weights from 11 inoculations (minimum of 20 seeds each) were used to calculate the average amount of inoculum applied by this procedure.

For the infested sand method, seedlings were planted so that they grew through sand artificially infested with teliospores. One untreated seed of either Pac-1 or C/W-74 safflower was placed on soil in pots as described above, and a 2.5-cm-diameter plastic column was placed over each seed. The column was filled 2.5 cm high with sand artificially infested with either 0.1 or 1.0 mg of dry spores of *P. carthami*. The remainder of the soil surface in each pot was covered with 2.5 cm of noninfested sand while the plastic column was in place. The column was removed, leaving 12.25 cm³ of infested sand directly over the seed. Twenty pots were set up for each combination of cultivar and treatment. Experimental design and data analysis are described below. The experiment was conducted with each isolate of *P. carthami*.

Wet spore inoculations were initiated to improve quantification and control of the inoculation process. Inoculum was suspended in a 0.1% water agar plus wetting

agent (WAWA) carrier. Wetting agents used in these experiments were either a silicone-polyether copolymer (Silwet L-77; Loveland Industries, Greeley, CO) or Tween 20 at 0.2 and 0.125% (vol/vol), respectively. The addition of water agar to the carrier kept the teliospores in suspension, facilitating both quantification of teliospores and inoculation of seedlings (7). Two-day-old safflower seedlings were inoculated with a 1- μ l drop of spores in WAWA, planted, and grown as described earlier.

Inoculum was delivered as weight of spores per seed or seedling in the initial wet spore inoculations. For the remaining series of experiments, inoculations were quantified in terms of the number of teliospores per seed or seedling. Three to 5 mg of spores was mixed in 100 μ l of WAWA. Concentration of teliospores was estimated in a 10⁻² dilution of the stock suspension by counting the teliospores in five 1- μ l drops on a glass slide with a microscope at $\times$ 100 magnification. The number of teliospores estimated per microliter of stock suspension was adjusted to the highest desired treatment concentration by diluting with WAWA, and dilutions were made from this to obtain the remaining spore concentrations. Verification of teliospore counts was done with 1- μ l drops as described. Minor adjustments were made, if necessary, in the volume of WAWA delivered to each seedling on the basis of verification counts. Teliospore germination was determined by placing a minimum of five 1- μ l drops of treatment suspension on 1.5% water agar plates containing a germinating safflower seed (8) and counting spore germination percentages determined after 1 week.

Experiments. Following preliminary tests with dry spore or infested sand methods, comparative experiments were conducted with each isolate of *P. carthami*. Twenty pots of Pac-1 and CW-74 were used with each treatment combination.

Two experiments, one for each isolate, were conducted to determine the importance of inoculum placement on seedlings and disease development on safflower plants. Pac-1 seedlings each were inoculated with 20 μ g of spores in a 1- μ l drop of WAWA at four locations along the hypocotyl and root (Fig. 1). Drops were allowed to dry before seedlings were planted. Ten seedlings were inoculated for each combination of isolate and treatment. Based on results from these experiments, seedlings in subsequent studies were inoculated on the hypocotyl at the base of the cotyledons (Fig. 1).

To measure the effects of isolate, inoculum concentration, cultivar, and watering regime on damage by *P. carthami*, 10 seedlings each of Pac-1 and UC-41 were inoculated with 1- μ l drops containing 0, 15, 60, or 240 teliospores of each isolate. Seedlings were planted and grown at 12 to 14°C with misting twice per week during the first 3 weeks. Plants then were moved to a greenhouse maintained at 21 to 25°C, separated into two groups, and either watered on a daily basis (no wilting) or watered after control plants began to wilt (reversible wilting).

Experimental design and data analysis. Each experiment was run as a randomized complete design with pots (one plant per pot) as replicates. Data were recorded on emergence, post-emergence damping-off, plant area diseased (percentage of hypocotyl), pustule development (0 to 4 rating in which 0 = no pustule and 4 = heavy sporulation), plant height (cm), leaves (number), and plant fresh and dry weights (g), for each study. Weighted least square means were calculated and data evaluated with the SAS (SAS Institute, Cary, NC) general linear models (GLM) procedure. Sources of variation included main effects and their interactions. Mean separation was based on comparing probability for differences (SAS, PDIFF procedure) between weighted least square treatment and interaction means. Treatment and interaction means were considered significantly different at $P \leq 0.05$. Interactions identified from the GLM procedure were clarified on the basis of the statistical separation of interaction means. Categorical data for disease ratings from the inoculum concentration experiment were subject to non-parametric analysis (SAS, NPARIWAY procedure) for the main effect, watering regime.

RESULTS

Dry spore versus sand column inoculation of safflower. The amount of inoculum estimated to adhere to each seed rolled in dry spores was 0.219 mg (CI [$P = 0.05$] = ± 0.176 mg, $n = 11$ experiments) for both the MEX and CAL isolates. Dry weight of Pac-1, but not C/W-74, was significantly less than the

Fig. 1. Locations for inoculation of the primary root of a 2-day-old safflower seedling for data presented in Table 2. Protocol proposed involves inoculations on the hypocotyl at the base of the cotyledons ("Cotyledon Base").

Table 1. Weighted least square means for dry weight and height from inoculation of two safflower cultivars^w, Pacific-1 (Pac-1) or Cal/West-74 (C/W-1), with teliospores of *Puccinia carthami*^w

Inoculation method ^x	Dry weight (g)				Height (cm)			
	CAL		MEX		CAL		MEX	
	Pac-1	C/W-74	Pac-1	C/W-74	Pac-1	C/W-74	Pac-1	C/W-74
Control	0.91 a ^y	0.73 a	0.55 a	0.54 a	57.9 ab	41.8 a	46.7 ab	22.3 a
Sand, 0.1 mg/plant	0.96 a	0.71 a	0.55 a	0.43 b	57.8 ab	41.5 a	38.9 b	16.2 b
Sand, 1.0 mg/plant	0.81 a	0.055 a	0.55 a	0.46 ab	60.8 a	43.2 a	42.4 ab	21.9 a
Dry @ 0.16 mg/plant	0.24 b	0.61 a	0.15 b	0.50 ab	49.5 b	32.5 b	56.7 a	23.0 a
Reduction (%) ^z	(73.6)	(16.4)	(72.7)	(7.4)				

^wCultivars were either very susceptible (Pac-1) or moderately susceptible (C/W-74) to the foliar phase of safflower rust. Data represents results from two separate experiments, one with a field isolate from California (CAL) and the other with a Mexican (MEX) isolate

^x Plants were inoculated either directly by dusting seeds with teliospores (Dry) or indirectly by planting inoculated seeds under a column of sand infested with teliospores (Sand).

^y Means followed by the same letter in each column are not statistically significantly different ($P_p = 0.05$), based on the t test option in the general linear models procedure (SAS Institute, Cary, NC). Means are the average of 20 observations.

^z Calculated, for dry weight only, by: [Dry weight (Control) – Dry weight (Dry) / Dry weight (Control)] $\times$ 100.

controls by an average of 73.2% for both isolates of *P. carthami* (Table 1), and the cultivar $\times$ treatment interaction was significant ($P = 0.001$) for each isolate. Plant heights were significantly less for both cultivars inoculated with dry spores of the CAL, but not the MEX, isolate. The cultivar $\times$ treatment interaction for either isolate was not significant for plant height data.

Plant dry weights and heights were not less than controls when seedlings grew through sand columns infested with teliospores of the CAL isolate of *P. carthami*. The mean dry weight of C/W-74 in the MEX isolate at 0.1 mg treatment was significantly less than the controls, by 20.3%, and plant heights for both Pac-1 and C/W-74 were significantly less than controls in the same treatment, by 16.7 and 27%, respectively.

Wet spore inoculation of seed; placement of inoculum. Greatest reductions in both plant dry weights (73%) and heights (52%) occurred when the drop of inoculum was placed on the hypocotyl at the base of the cotyledons (Table 2). The amount of damage was inversely proportional to distance from the cotyledons for inoculation, and there was no reduction in either dry weight or height when inoculum was placed in or near the root hairs (Table 2).

Wet spore inoculation of seed; inoculum concentration and watering regime. The main effect of watering regime was not significant ($P = 0.264$ and 0.124 for dry weight and height, respectively) following inoculation of both safflower cultivars with each isolate at different concentrations of teliospores.

A significant interaction was noted for inoculum concentration $\times$ cultivar ($P = 0.020$ and 0.015 for dry weight and height, respectively). Dry weight of Pac-1 inoculated with 240 teliospores per plant was reduced by 57% (Table 3) compared with a 27% reduction of UC41. Similar differences between the cultivars were noted for plant height data (Table 4).

The interaction of inoculum concentration $\times$ isolate also was significant for the two variables ($P = 0.009$ and 0.037 for dry weight and height, respectively). Dry weight of safflower inoculated with the MEX isolate at 240 teliospores per plant was reduced by 57% (Table 3) compared with a 23% reduction with the CAL isolate. These differences also were reflected in the plant height data (Table 4).

Although watering regime did not cause significant effects on yield components (plant dry weight and height), disease rating and plant area diseased were greater when plants were allowed to wilt before watering. This was significant for disease ratings only at the lower doses of teliospores (15 and 62.5 per seedling) and when plants of either cultivar were inoculated with the CAL isolate (Table 5), based on

the Kruskal-Wallis test (chi-square approximation). Plant area diseased also was significantly greater when plants were allowed to wilt before watering (31.2% for

both cultivars), compared with 21.0% when plants were not allowed to wilt ($P = 0.001$, paired t test for data sorted by cultivar and treatment).

Table 2. Weighted least square means of dry weights and heights from the safflower cultivar, Pacific-1, 6 weeks after inoculation with teliospores of field isolates of *Puccinia carthami* placed at different locations on 2-day-old seedlings^w

Location of inoculations ^x	Dry weight (g)		Height (cm)	
	CAL	MEX	CAL	MEX
Control	3.4 a ^y	2.2 a	47.9 ab	42.2 a
Within the root hairs	3.4 a	1.5 a	54.6 a	33.0 a
Hypocotyl at the root hairs	3.0 ab	1.9 a	50.8 ab	40.1 a
Hypocotyl	1.9 b	1.5 a	34.8 b	29.6 ab
Base of cotyledons	0.9 c	0.6 b	22.0 c	20.2 b
Reduction (%) ^z	(73.5)	(72.7)	(54.1)	(52.1)

^w Data represents results from two separate experiments, one with an isolate from California (CAL) and the other with a Mexican (MEX) isolate

^x Illustrated in Figure 1. "Hypocotyl at the root hairs" = "Root Hair Interface" in Figure 1.

^y Means followed by the same letter in each column are not statistically significantly different ($P = 0.05$), based on the t test option in the general linear models procedure (SAS Institute, Cary, NC). Means are the average of 10 observations.

^z Calculated, for dry weight only, by: [Dry weight (Control) – Dry weight (Dry) / Dry weight (Control)] $\times$ 100.

Table 3. Weighted least square means for dry weights of two safflower cultivars, Pacific-1 (Pac-1) and UC-41, after inoculation with a 1- μ l drop containing a known number of teliospores of *Puccinia carthami* isolates from California or Mexico placed on the hypocotyl near the base of the cotyledons of 2-day-old seedlings^w

Inoc Conc (T)	Isolate (I)								
	California			Mexico			Both		
	Cultivar (C)								
	Pac-1	UC-41	̄	Pac-1	UC-41	̄	Pac-1	UC-41	Inoc
			(I*T) ^x			(I*T)	(-----T*C-----)		Conc
0.0	2.05a ^y	2.24 a	2.14 a	2.35 a	2.77 a	2.56 a	2.20 a	2.50 a	2.35 a
15.0	1.96 a	2.08 a	2.02 ab	2.32 a	2.07 ab	2.20 a	2.16 a	2.07 ab	2.11 a
60.0	1.27 b	1.87 a	1.57 c	1.34 b	2.03 ab	1.69 b	1.31 b	1.95 b	1.62 b
240.0	1.24 b	2.04 a	1.64 bc	0.62 c	1.59 b	1.11 c	0.94 c	1.82 b	1.38 b
Reduction (%) ^z			(23.4)			(56.6)	(57.3)	(27.2)	

^w Cultivars were either very susceptible (Pac-1) or moderately susceptible (UC-41) to the foliar phase of safflower rust. Inoc Conc = Inoculation concentration.

^x Significant interaction occurred between main effects.

^y Means followed by the same letter in each column are not statistically significantly different ($P = 0.05$), based on the t test option in the general linear models procedure (SAS Institute, Cary, NC). Means are the average of 20 observations.

^z Calculated by: [Dry weight (Control) – Dry weight (Dry) / Dry weight (Control)] $\times$ 100.

Table 4. Weighted least square means for plant heights of two safflower cultivars^w, Pacific-1 (Pac-1) and UC-41, after inoculation with a 1- μ l drop containing a known number of teliospores of *Puccinia carthami* isolates from California or Mexico placed on the hypocotyl near the base of the cotyledons of 2-day-old seedlings

Inoc Conc (T)	Isolate (I)								
	California			Mexico			Both		
	Cultivar (C)								
	Pac-1	UC-41	̄	Pac-1	UC-41	̄	Pac-1	UC-41	Inoc
	(I*T) ^x			(I*T)			(-----T*C-----)		
0.0	37.5 a ^y	45.6 a	41.6 a	36.4 a	47.1 a	41.8	37.0	46.4	41.7 a
15.0	32.5 ab	46.2 a	39.4	35.6 a	41.0 a	38.3	34.0	43.6	39.4 a
60.0	27.2 a	44.0 a	35.6	23.2 a	44.3 a	33.8	25.2	44.2	34.7 b
240.0	30.2 ab	45.4 a	37.8	14.8 c	41.2 a	28.0	22.5	43.3	33.0 b
Reduction (%) ^z			(9.1)			(33.0)	(39.2)	(6.7)	

^w Cultivars were either very susceptible (Pac-1, Pacific-1) or moderately susceptible (UC-41) to the foliar phase of safflower rust. Inoc Conc = Inoculation concentration.

^x Significant interaction occurred between main effects.

^y Means followed by the same letter in each column are not statistically significantly different ($P = 0.05$), based on the t test option in the general linear models procedure (SAS Institute, Cary, NC). Means are the average of 20 observations.

^z Calculated by: [Dry weight (Control) – Dry weight (Dry) / Dry weight (Control)] $\times$ 100.

DISCUSSION

A procedure for the quantitative inoculation of safflower seedlings with teliospores was selected that involves inoculation of 2-day-old safflower seedlings each with a known number of teliospores in a 1- μ l drop of WAWA on the hypocotyl at the base of the cotyledonary leaves. Seedlings should be planted under sand, given a cool (12 to 15°C) environment (12) with limited watering for 3 weeks after inoculation, and then a warmer (21 to 25°C) environment for 2 to 3 weeks. Based on yield component data, watering regime was not a factor after emergence, so normal watering of plants is recommended after they emerge.

Disease incidence was both lower and inconsistent when inoculated seeds were planted under soil (data not shown) or when noninoculated seedlings grew through teliospore-infested sand. Halfon-Meiri (6) has shown that the use of teliospore-infested soil resulted in less disease than when contaminated seeds were used. The lower amounts of disease may be due to limited exposure to inoculum (lower concentration and physical separation), soil moisture, or aeration. The procedure described in this paper minimizes these factors as detriments by the use of direct inoculation of seedlings where the plant is most vulnerable, overlaying inoculated seed with sand, and controlling watering until the seedlings have emerged. The use of sand and restricting water before seedling emergence allows for good aeration of the teliospores under moist conditions. Klisiewicz (9) has shown the detrimental effects of too much moisture and the importance of aeration on germination of *P. carthami* teliospores.

Development of this procedure is pivotal to completing the risk assessment of *P. jaceae* for biological control of YST. Side-by-side comparative greenhouse studies are very useful to facilitate judgments about risk under expected field conditions (3,4). In earlier comparisons in the greenhouse, it

was clear that *P. jaceae* urediniospores caused, at most, only limited infections of safflower foliage. Relative to the infection from *P. carthami*, infections from *P. jaceae* were insignificant, and *P. jaceae* could not be maintained on safflower foliage under optimal greenhouse conditions (1). Foliar infections of safflower caused by *P. carthami* in the field result in yield losses only in seasons when there is sufficient moisture for leaf infection during flowering (20). The seedling phase of safflower rust, by contrast, is potentially more damaging and capable of causing significant annual production losses if susceptible cultivars are planted and practices to minimize seed and soil contamination are not followed (18). Teliospore-infested seed and soil may lead to the girdling and death of seedlings (13,17). What remains is the test of safflower seedling susceptibility to infection from *P. jaceae* teliospore inoculations.

The evaluation of *P. jaceae* teliospores on safflower also requires a supply of viable inoculum. This has been limiting until recently, when a procedure was developed to prime teliospores of *P. jaceae* to germinate (2). Plans are to conduct the comparative quantitative inoculation experiments with the protocol developed from this research. With this protocol, it will be possible to control and validate the number of viable teliospores applied for each pathogen. Previously described procedures for safflower inoculations are only semiquantitative, and involve either dusting seeds with teliospores at the rate of 1.0 g per kg seed (18), treating 15 g of safflower seeds previously moistened with 5% gelatin with 0.4 g of teliospores (19), submerging 2-day-old seedlings in a suspension of teliospores (12), or pouring a suspension of teliospores over emerging safflower seeds in soil flats (9,16). These methods also require relatively large quantities of teliospores, which are easily obtained for *P. carthami*, but which may be limiting for *P. jaceae*.

The procedure developed for this study is similar to one described by Toussoun et al. (14) to inoculate bean stems with *Fusarium solani*. A capillary pipette was used to place drops of macroconidia on detached bean stems that were then incubated in a moist chamber and evaluated after 4 days of inoculation. Their procedure was semiquantitative and the placement of inoculum in their study was less critical. In the present study, successful inoculation of safflower seedlings depended on both the amount and the placement of inoculum. Additionally, the teliospore inoculation procedure differs from the capillary pipette procedure by the use of 0.1% water agar both to keep spores in suspension during quantification and to hold spores in place on the seedling, use of a wetting agent to facilitate suspension of inoculum, and application of a smaller volume of inoculum (1- μ l versus 5- μ l drops) delivered to the plant.

All three safflower cultivars were damaged by infection from *P. carthami* in this study, but differences in cultivar susceptibility were noted. This is based on the statistically significant interactions between cultivar and treatments (Tables 1, 3, and 4) where Pac-1 had greater losses in terms of dry weight and height than the other two cultivars. These results support findings from other studies (12,16,18,20). Evidence from this study also supports data suggesting a correlation between susceptibility of safflower seedlings (from teliospore infestations) and older plants (from urediniospores) (12,20). In the present study, both UC-41 and C/W-74 were less susceptible than Pac-1 to infection by *P. carthami*. The same order of susceptibility was reported for these cultivars in an earlier study on foliar infections by *P. carthami* (1). In the earlier cultivar study with urediniospores, cultivars least susceptible to *P. carthami* also were least susceptible to *P. jaceae* (1). The hypothesis concerning relative cultivar susceptibility to *P. carthami* and *P. jaceae* now can be tested in studies with teliospores.

Results from the inoculum concentration experiment suggest there is no interaction between yield components (plant height, fresh and dry weights) and water stress after inoculation. This supports findings from an earlier study (12). Although yield components were not affected by watering regime, there was evidence that disease, based on ratings or estimates of plant area infected, was greater when plants were allowed to wilt before watering. Differences noted for watering regime occurred only where disease pressure was lowest, i.e., after inoculation by the CAL isolate at the two lowest teliospore concentrations (15 and 62.5 per seedling). The CAL isolate was less aggressive than the MEX isolate in this study.

Effects of watering on the amount of disease (versus yield) resulting from *P.*

Table 5. Mean disease ratings^a and the mean Wilcoxon sums^v for disease 6 weeks after inoculating the primary roots of 2-day-old safflower seedlings with *Puccinia carthami* teliospores^w

Isolate ^y	Cultivar ^z	Watered ^x			Watered ^x			Kruskal-Wallis test ^v	
		n	Rating	Wilcoxon	n	Rating	Wilcoxon	Chi-sq	P > Chi-sq
CAL	Pac-1	19	2.00	14.2	20	4.00	25.5	15.46	0.001
CAL	UC-41	19	1.68	16.2	20	3.20	23.6	5.76	0.016
MEX	Pac-1	19	3.37	18.1	18	4.00	20.0	.098	0.323
MEX	UC-41	20	2.60	19.0	20	3.20	22.0	1.10	0.294

^a Disease ratings are from 0 = no macroscopic symptoms to 4 = large pustules, elongated, and twisted hypocotyls or plant death.

^v Calculated for nonparametric data (NPAR1WAY, SAS Institute, Cary, NC).

^w Data are combined from the 15 and 62.5 teliospores per seedling treatments only; differences were not significant in the controls or in the 240 teliospores per seedling treatments.

^x Plants either were watered on a regular basis (Watered) or were allowed to sustain a reversible wilt before watering (Wilted).

^y Isolates are designated by location of source. CAL = a Californian field isolate. MEX = a Mexican field isolate.

^z Cultivars were either very susceptible (Pac-1, Pacific-1) or moderately susceptible (UC-41) to the foliar phase of safflower rust.

carthami teliospore inoculations were not reported in earlier studies on safflower rust, suggesting that the inoculation procedure proposed in the present study brings a much greater level of control and sensitivity to investigations of this disease. Otherwise, the proposed procedure compares favorably with other inoculation procedures (9,12,16–19). For these reasons, the procedure developed from this study will be a valuable part in completing the risk assessment of *P. jaceae* for biological control of YST.

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