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Effects of the rust *Puccinia jaceae* var. *solstitialis* on *Centaurea solstitialis* (yellow starthistle) growth and competition

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ABSTRACT

The rust fungus *Puccinia jaceae* var. *solstitialis* was introduced to California in 2003 for biological control of yellow starthistle (*Centaurea solstitialis*). To test its effectiveness under field conditions, we examined biomass production, chlorophyll levels, and seedhead production of yellow starthistle (YST) infected with *P. jaceae*. We also evaluated the effect of *P. jaceae* on the competitive ability of YST grown with wild oat (*Avena fatua*), a widespread winter annual grass commonly associated with YST infestations. Chlorophyll levels were reduced by over 50% in severely rust-infested YST leaves. *P. jaceae* had no effect on growth or reproductive variables in monoculture plots, but caused a modest reduction in YST performance in the competition experiment. In this study, infected plants had fewer leaves than uninfected plants and slightly reduced rosette diameters. *P. jaceae* also decreased YST biomass from 3.18 kg m^{-2} in non-inoculated competition plots to 2.62 kg m^{-2} in inoculated plots over both years of the experiment. Although not significant, there was a trend towards reduced seedhead production in the inoculated plots. These results indicate that *P. jaceae* may have some negative effects on YST growth, especially under conditions of interspecific competition. However, the effects of *P. jaceae* appear to be of minor biological significance and are unlikely to cause major declines in YST populations statewide.

1. Introduction

Yellow starthistle (*Centaurea solstitialis* L.) is one of the worst invasive plants in the western United States (DiTomaso et al., 2006b). In California alone, it is estimated to cover 5.8 million hectares of land and continues to spread (Pitcairn et al., 2006). Yellow starthistle (YST) is native to the Mediterranean climates of Eurasia (Maddox, 1981) and is thought to have been introduced to California in contaminated alfalfa seed (Roche and Thill, 2001; Gerlach, 1997; Maddox, 1981; Robbins et al., 1941) sometime after 1824 (Maddox, 1981). Since then it has become a widespread problem in rangelands, wildlands, and roadsides, where it can displace native vegetation (see DiTomaso et al., 2006b for review), provide poor late season forage (Eagle et al., 2007), alter soil water dynamics (Enloe et al., 2004), and poison horses (Cordy, 1978). Multiple strategies have proven to be effective in controlling YST including mowing (Benefield et al., 1999; Thomsen et al., 1994), grazing (Thomsen et al., 1994), burning (DiTomaso et al., 2006a, 1999a), and chemical treatments (DiTomaso et al., 1999b). However, given the expansive distribution of the plant (Pitcairn et al., 2006), these control techniques are not always cost effective or feasible (Bruckart, 1989).

The first insect biological control agent for yellow starthistle was introduced in 1969. Since then, five additional insect agents have been released (Pitcairn et al., 2004). All of these agents attack starthistle seedheads. Of these six insect agents only two, *Eustenopus villosus* Boheman (hairy weevil) and *Chaetorellia succinea* Hering (false peacock fly), are both widespread and at least moderately effective (see DiTomaso et al., 2006b, for review). These agents may have reduced YST populations in localized areas (Woods et al., 2004b), although there has not been a large-scale reduction in YST populations statewide (Pitcairn et al., 2006).

A new biological control pathogen, *Puccinia jaceae* Otth var. *solstitialis* (yellow starthistle rust), was approved for release in California in 2003 (Woods et al., 2004a). Rusts are considered potentially effective biological control agents given their natural dispersal mechanism (wind) and their narrow host range (Shishkoff and Bruckart, 1993; Watson and Clement, 1986). A similar rust, *Puccinia chondrilla* Bubak and Syl., has proven to be an effective control agent on rush skeletonweed (*Chondrilla juncea* L.) populations in some areas of the western United States (Supkoff et al., 1988).

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The goal of the YST pathogen biological control program is to provide an additional stress that will complement the activity of the seed feeding insect bioagents (Turner et al., 1995).

Puccinia jaceae was originally collected in Turkey (Bruckart, 1989) and is the first biocontrol pathogen to be released in the United States under the modern USDA-APHIS permitting process (Fisher et al., 2007). *Puccinia jaceae* is a macrocyclic and autoecious obligate pathogen (Savile, 1970) that attacks the vegetative parts of the plant, particularly the leaves (Woods and Villegas, 2004; Turner et al., 1995). In potted plants with multiple inoculations, Bennett et al. (1991) found that *P. jaceae* reduced YST shoot biomass by 50% and root biomass by 40%. Other greenhouse studies have also shown *P. jaceae* to decrease root biomass and increase leaf senescence (Shishkoff and Bruckart, 1993).

Although there have been extensive greenhouse experiments (Shishkoff and Bruckart, 1996; Bennett et al., 1991; Bruckart, 1989), there have been few published studies on the performance of *P. jaceae* under controlled field conditions (Fisher et al., 2007; Woods and Popescu, 2007). Fisher et al. (2007) conducted a field study examining the optimal conditions for establishing *P. jaceae* on YST. Swope (2009) examined the interaction of *P. jaceae* with the YST biocontrol insect *Eustenopus villosus* and found that insect attack rates were higher on rust-infected plants.

The objectives of this study were to evaluate the effect of *P. jaceae* var. *solstitialis* on a variety of YST growth parameters, including photosynthesis, growth, and reproductive output, and to determine the influence of the rust on the competitive interaction between YST and wild oat (*Avena fatua* L.) under field conditions. Wild oat is a common introduced grass in rangelands, and is considered a desirable forage species that often competes with YST. We accomplished these objectives through replacement series competition studies and YST monoculture (density) experiments.

2. Materials and methods

Experiments were performed in 2006 and repeated in 2007 at the University of California-Davis Plant Sciences Field Station in Yolo County. The soil at the station is Yolo fine sandy loam. In 2005–2006 and 2006–2007, the station received 59 and 21 cm of rainfall, respectively (http://www.ipm.ucdavis.edu/WEATHER/abtwxvars.html). Yellow starthistle and wild oat seeds were collected within a 1.6 km (1 mile) radius of the field station during the summer prior to planting. Seeds were stored in paper bags under ambient indoor temperatures (20–24 °C, ~25% relative humidity) for 4–7 months until the initiation of the experiments.

2.1. Seedlings

Seeds of YST and wild oat were planted in 200-plug 2.54 cm^2 Speedling® flats (Speedling, Inc., Sun City, FL) in a 50:50 mixture of field soil (Yolo fine sandy loam) and potting soil (30:30:30; peat moss, perlite, and vermiculite). All planting soils were shredded and sterilized in an autoclave. We planted one to five seeds per plug in December 2005 to early January 2006, and early January 2007. The flats were stored in a greenhouse at 20/12 °C (day/night) as per Buhler et al. (1999), and irrigated using subirrigation trays. Seedlings were moved to a lath house at the 2–5 leaf stage, three weeks before planting. All plants were fertilized as necessary.

2.2. Field site

The 2006 and 2007 experimental sites were located approximately 200 m apart to prevent residual infection of *P. jaceae* spores from the previous year. Prior to planting, the sites were sprinkler irrigated to promote weed germination, followed by harrowing several weeks later. The 2006 study area was pretreated with Roundup Pro® (glyphosate, Monsanto Company, St. Louis, MO) to kill emerged weeds, and the 2007 area was pretreated with glyphosate and Surflan® AS (oryzalin, United Phosphorus Inc., Trenton, NJ). In both years, the field sites were fenced to prevent mammalian herbivory.

2.3. Experimental design

Two types of experiments, a monospecies study and a replacement series competition study, were used to assess the impact of *P. jaceae* on YST. Both designs used 1 m × 1 m plots, separated by 1 m bareground buffers on all sides in 2006 and 2 m buffers in 2007.

In the monospecies study, YST was established at three densities (5, 16, and 64 plants m^{-2}) to examine the effects of *P. jaceae* on a variety of growth parameters in intraspecific competitive interactions. Inoculated and non-inoculated treatments were included for each density. In the five-plant density plots, four plants were planted in each corner, and one plant in the middle of the quadrat. Only the center plant was harvested and evaluated to eliminate edge effects. The 16-plant plots were planted in a 30-cm grid, with the center four plants harvested and evaluated. The 64-plant plots were planted in a 13-cm grid and the center 16 plants were harvested and evaluated. The experiment was established as a randomized complete block design with five blocks in 2006 and four blocks in 2007.

In the replacement series study (Harper, 1977; de Wit, 1960), we evaluated the effect of *P. jaceae* on competitive interactions between YST and wild oat, a robust winter annual grass common in California rangelands and grasslands and often occurring in areas with YST. Plants were established at a constant density of 36 plants m^{-2} using a 20-cm grid, with varying proportions of YST:wild oat. In 2006 we used YST:wild oat ratios of 0:100, 25:75, 50:50, 75:25, and 100:0. More extreme ratios (YST:wild oat = 10:90 and 90:10) were added in 2007. Eight plots of each proportion were established, and half were inoculated with *P. jaceae* in a randomized complete block design with four blocks. To minimize edge effects, only the inner 16 plants of each plot were harvested and evaluated, comprising 16, 12, 8, 4, 1, and 0 YST plants at the 100:0, 75:25, 50:50, 25:75, 10:90, and 0:100 ratios, respectively (and the reverse for wild oat).

2.4. Outplanting

In 2006, the replacement series experiment was transplanted to the field at the end of January, and the density experiment was transplanted in the first week of February. In 2007, both experiments were transplanted the first week of February when plants were at the 3–6 leaf stage. In 2006, dry, warm conditions in February necessitated 50 mm of supplemental irrigation through sprinklers; in the drought year of 2007, approximately 90 mm of irrigation was applied from December to March. Both the plots and the buffers were hand weeded weekly until harvest. There was 10% YST and 1% wild oat mortality in 2006 and 3% YST and 4% wild oat mortality in 2007. Dead plants were replaced prior to inoculation with same-sized seedlings maintained in the lath house. No dead plants were replaced after inoculation in any plots.

2.5. Inoculation

The plots were inoculated at the beginning of March in both years and reinoculated at the end of March in 2006. Fisher et al. (2007) found March inoculations to be optimal for *P. jaceae* infection.

Puccinia jaceae uridiniospores were provided by the California Department of Food and Agriculture (CDFA) Biocontrol Program

in Sacramento. Spores were stored in a freezer at −70 °C and transferred to a standard freezer for approximately a week before inoculation. We applied spores in a solution of 500 mg spores l^{-1} water, with an emulsifier, Tween-20® (polyoxyethylene sorbitan monolaurate, EM Sciences, Gibbstown, NJ), at a rate of 0.6 ml l^{-1}. A CO_2 backpack sprayer and two-nozzle boom were used to apply the spores in 923 l hectare^{-1} (100 gal acre^{-1}) of spray solution, which we previously found to be an effective 'spray-to-wet' rate for small plants. Control plots were sprayed with the same solution and volume, minus the *P. jaceae* spores.

In previous studies, infection levels were greatest when newly inoculated plants were subjected to 12 h of dew (Fisher et al., 2007; Woods et al., 2004a; Bennett et al., 1991). Therefore, treated plots were covered with plastic tents following inoculation to maintain high humidity. Tents were removed the following morning at least 16 h after treatment.

The time from *P. jaceae* inoculation until the appearance of pustules ranged from 10 to 21 days. As such, plots were evaluated after three weeks to determine whether reinoculation was required. In 2006, all plots were reinoculated, whereas only four plots, all in the replacement series, required reinoculation in 2007.

2.6. Fungicide

When non-inoculated plots showed early signs of infection, we applied Quadris® (azoxystrobilin, Syngenta Crop Protection, Greensboro, NC) to control plots in order to minimize infection. Non-inoculated plots were sprayed once in May 2006 and twice in May 2007. In 2007, a second set of non-inoculated YST monoculture plots was established 10 m from the experiment to test for the effects of the fungicide on YST growth and reproduction. No differences were found in any measured parameter (data not shown). A subsequent greenhouse experiment was also established in 2007 to test for fungicide effects on YST, with similar results as the field study (data not shown).

2.7. Evaluations

2.7.1. Infection and foliage growth

After symptoms began to appear, we collected infection incidence and severity data at three to four week intervals until harvest. Measurements were taken on four plants per plot early in the season when the plants were small and three plants per plot on larger plants later in the season. In 2007, the radii of YST rosettes were measured until the bolting stage in mid-May. We counted total leaf numbers on each plant and assigned plants to classes of 10–30 leaves, 31–50 leaves, and >51 leaves. We defined infection incidence by determining the proportion of infected leaves out of total leaves, then categorized plants into infection incidence classes of 1 = 0–25%; 2 = 26–50%; 3 = 51–75%; and 4 = 76–100% of total leaves infected. For each plant, the three most infected leaves were rated for severity according to an infection key developed by Fisher and Woods (unpublished), where 0 = no pustules, 1 = 1–4 pustules per leaf, 2 = 5–15 pustules per leaf, 3 = >16 pustules per leaf but not uniform, 4 = numerous pustules per leaf with moderate uniformity, and 5 = numerous pustules per leaf uniformly dispersed throughout. For the purpose of analysis, an infection index was created by multiplying the infection class (1–4) and the averaged infection ratings (0–5). The resulting index values ranged from 0 to 20, with 0 being no infection, and 20 being all measured leaves infected at the highest infection rating.

2.7.2. Chlorophyll

A Minolta SPAD 502 Meter® (Spectrum Technologies, Inc., Plainfield, IL) was used to estimate YST leaf chlorophyll levels (Ichie et al., 2002; Richardson et al., 2002). The Minolta SPAD is a hand-held, non-destructive way of estimating chlorophyll content in leaves (Richardson et al., 2002). Chlorophyll measurements were taken twice each year between April 26 and May 25. We sampled leaves with a range of infection rates in six of the inoculated plots, including four 100:0 plots and two 75:25 plots. In each plot, we measured four to five leaves covering the full range of the Fisher and Woods infection rankings. Overall, fewer than 10 leaves were found with the highest infection rating (level 5). Five measurements were taken per leaf and then averaged using the SPAD (according to van den Berg and Perkins, 2004). Measured leaves were in the upper third of the canopy and all measurements were taken in full sunlight between 1100 and 1500 h.

2.7.3. Data processing

Above-ground biomass was harvested in July of both years when YST plants were at full flower. The center portion of each plot (replacement series experiment was 0.64 m^2; density experiment was 0.27 m^2 for 64 plants m^{-2}, 0.36 m^2 for 16 plants m^{-2}, and 0.25 m^2 for 5 plants m^{-2}), minus the edges, was clipped and values were converted to biomass per m^2. Total numbers of living and dead plants were recorded for each species in each plot. Dead plants were not used in analysis. Harvested plants were dried outdoors for approximately two weeks before weighing. To estimate the total number of YST seedheads per plot, seedheads were counted in a 150 g subsample from each plot. Only seedheads in the spiny stage with four or more spines fully extended at 90° were included in the count. In order to estimate seed reproductive potential (Pitcairn et al., 1998), we measured diameters of 20 seedheads per plot.

For the wild oat plants, seed weights were estimated based on seed collected from bagged plants outside the plots (data not shown). A calculated relationship was determined for spikelet number and caryopses weight. These values were added back to the wild oat weights in each plot (based on spikelet numbers per plant) to account for caryopses which dispersed prior to harvest.

2.8. Data analyses

2.8.1. Growth and reproduction

In both experiments, biomass m^{-2}, seedheads m^{-2}, biomass plant^{-1}, and infection data were analyzed using ANOVA. Main rust effects and interaction terms (rust × treatments) were compared using pairwise contrast. Data were analyzed within and across years. For the replacement series, across-year comparisons were only performed for proportions used in both years. Radius and height data were analyzed only for 2007. Chlorophyll data were analyzed using ANOVA with YST:wild oat ratio and infection rating as the main effects. Interactions of *P. jaceae* and plant ratio effects were also determined using pairwise contrast. Chlorophyll differences by infection rating were determined using the Tukey test. Total leaf number class data were analyzed using ordinal logistic regressions. Leaf number data were analyzed by date, by year, and across all dates for both years. The replacement series design was unbalanced due to missing data for the 10:90 proportion planting in one block. The density experiment was also unbalanced due to a different number of blocks from one year to the next. All data were analyzed using JMP© (SAS Institute, 2005).

2.8.2. Competition indices

In order to evaluate the effects of *P. jaceae* on the competitive ability of YST, we determined relative yields for YST and wild oat, the relative crowding coefficient (RCC) *sensu* Harper (1977), and the relative yield totals (RYT) and aggressivity (Radosevich et al., 1997; McGilchrist and Trenbath, 1971). All values were analyzed within and across years using ANOVA. The 10:90 proportion was not used in across-year analyses. Main rust effects and

interactions between proportions and *P. jaceae* were analyzed using LS contrast. Relative yields for the replacement series graphs were compared using multi-response permutation procedures (MRPP), as performed by PC-ORD©, version 4.0 (MJM Software Design, Glenedon Beach, OR). MRPP is a non-parametric method for analyzing differences between groups (McCune and Grace, 2002). The replacement series graphs were interpreted according to Harper (1977). For both years combined, relative crowding coefficients were calculated two ways: (1) by using the raw data to calculate RCC values for each proportion in each block, then analyzing all of the data; and (2) by calculating averages for each proportion, and formulating the RCC using these averages. For individual years, RCCs were only calculated using the raw data as in method one.

3. Results

3.1. Infection levels

Originally, we expected that *P. jaceae* would not spread from the treatment zone in the first year of application. However, infection of non-inoculated plots occurred in both 2006 and 2007 (Fig. 1). Nevertheless, the inoculated plots always had significantly higher infection rates than the non-inoculated plots ($P < 0.0001$).

3.2. Chlorophyll

Higher levels of infection were expected to reduce the health and vigor of YST leaves. There was a significant reduction in leaf chlorophyll at the higher rust infection classes ($P < 0.0001$, Fig. 2). At the highest infection level (5), chlorophyll was reduced by 50% compared to uninfected leaves.

3.3. Density experiment, all years combined

In the density experiment, there were no differences between inoculated and non-inoculated plots for total biomass m^{-2}, biomass $plant^{-1}$, total seedheads m^{-2}, or seedhead diameter within or between years (data not shown). *Puccinia jaceae* also had no effect on total leaf number at any density (data not shown).

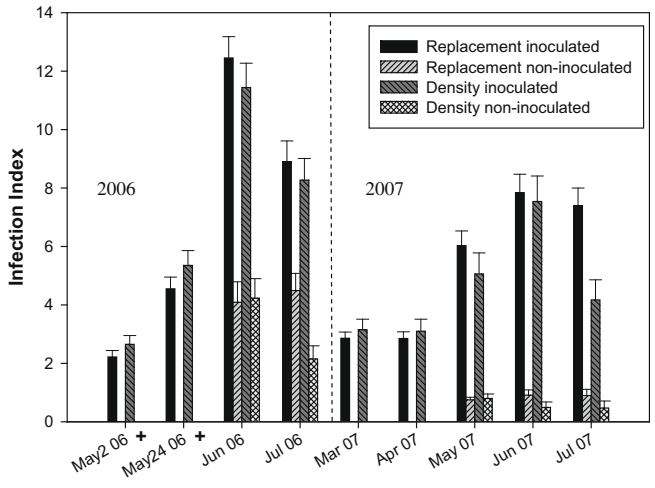

Fig. 1. Infection index means for the replacement series and density experiments for all dates (±SE). Dates with (+) indicate no data are available for non-inoculated plots. Infection index was created by multiplying the infection class (1–4) and the averaged infection ratings (0–5). The resulting index values ranged from 0 to 20, with 0 being no infection, and 20 being all measured leaves infected at the highest infection rating.

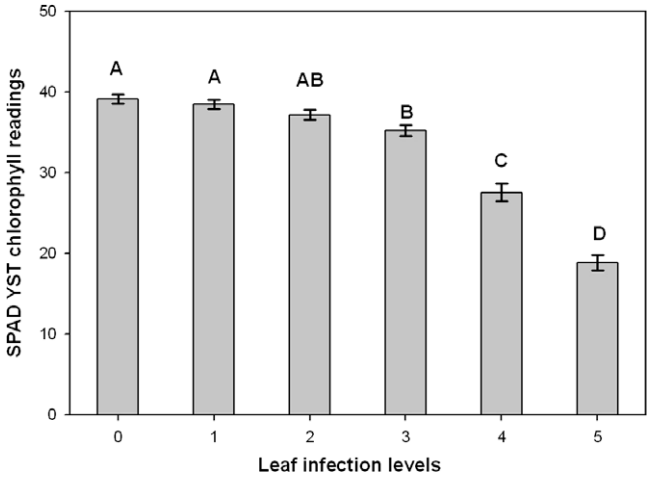

Fig. 2. Effects of *Puccinia jaceae* on chlorophyll levels at different infection rating classifications for 2006 and 2007 combined. Chlorophyll averages ($N > 8$) shown (±SE). Different letters represent significance at $P < 0.0001$. Leaf infection level was developed by Fisher and Woods (unpublished), where 0 = no pustules, 1 = 1–4 pustules per leaf, 2 = 5–15 pustules per leaf, 3 = >16 pustules per leaf but not uniform, 4 = numerous pustules per leaf with moderate uniformity, and 5 = numerous pustules per leaf uniformly dispersed throughout.

3.4. Replacement series experiment

3.4.1. Biomass

In the 2006 replacement series, there were no statistical differences between inoculated and non-inoculated plots for YST or wild oat biomass (Table 1). The combined data showed a similar mean YST biomass of 2.3 kg m^{-2} in inoculated plots and 2.4 kg m^{-2} in non-inoculated plots. In 2007, however, YST biomass was significantly higher in non-inoculated plots at the 50:50 and 100:0 proportions and, although not significant, was higher in non-inoculated plots at other proportions (Table 2). The combined data showed a significant reduction in total YST biomass and biomass per plant in inoculated plots concurrent with a significant increase in total wild oat biomass and biomass per plant. In 2007, YST biomass was reduced by 27% compared to untreated plants. Combining the data from 2006 and 2007, non-inoculated plants (3.2 kg m^{-2}) weighed 23% more than inoculated plants (2.6 kg m^{-2}) (Table 3). This was similar to the 17% increase in wild oat biomass in inoculated plots compared to non-inoculated. Data was combined across years, as both years showed similar variance. In the combined data, YST biomass per plant showed similar trends but no significant differences.

3.4.2. Seedheads

Seedhead numbers were estimated for each plot by subsampling the harvested biomass. At the 10:90 and 50:50 proportions (YST:wild oat), total seedheads m^{-2} in non-inoculated plots were significantly higher than in inoculated plots ($P < 0.10$, data not shown). However, no significant differences were found at other proportions. Overall data for 2006, 2007, and both years combined indicate a consistent trend towards higher seedhead numbers in non-inoculated plots (11–21% higher), but this trend was not significant ($P = 0.13–0.20$, Fig. 3). Seedhead diameters did not show any overall difference between inoculated and non-inoculated plots (data not shown).

3.4.3. Leaf number and rosette size

When plants were categorized into leaf number classes (10–30, 31–50, and >51), there was a significant 12% increase in the number of plants with >51 leaving in the non-inoculated plots ($P < 0.05$,

Table 1
Yellow starthistle and wild oat replacement series means in 2006. Values are given for the inoculated (*I*) and non-inoculated (*N*) plots at each proportion of YST to wild oat, and then combined for the main rust effect of all proportions. There were no statistical differences within species between *I* and *N* treatments for any proportion of the two species.

YST:wild oat proportions	0:100		25:75		50:50		75:25		100:0		Combined	
	I	*N*	*I*	*N*	*I*	*N*	*I*	*N*	*I*	*N*	*I*	*N*
YST biomass												
Total biomass (kg) m^{-2}	–	–	1.4	1.6	2.0	2.5	2.8	2.3	3.1	3.3	2.3	2.4
Biomass (kg) $plant^{-1}$ m^{-2}	–	–	0.44	0.42	0.27	0.31	0.27	0.21	0.23	0.24	0.30	0.30
Wild oat biomass												
Total biomass (g) m^{-2}	558	576	489	361	298	272	232	241	–	–	394	363
Biomass (g) $plant^{-1}$ m^{-2}	35	36	47	32	42	37	54	75	–	–	44	45

Table 2
Yellow starthistle and wild oat replacement series means in 2007. Values are given for the inoculated (*I*) and non-inoculated (*N*) plots at each proportion of YST to wild oat, and then combined for the main rust effect of all proportions. Statistical differences denoted as follows: $P < 0.10(*)$, $P < 0.05(**)$, $P < 0.01(***)$.

YST:wild oat proportions	0:100		10:90		25:75		50:50		75:25		100:0		Combined	
	I	*N*	*I*	*N*	*I*	*N*	*I*	*N*	*I*	*N*	*I*	*N*	*I*	*N*
YST biomass														
Total biomass (kg) m^{-2}	–	–	1.9	2.8	4.0	4.5	2.5	4.3^{***}	2.5	2.7	2.8	4.2^{**}	2.7	3.7^{***}
Biomass (kg) $plant^{-1}$ m^{-2}	–	–	1.92	2.84^{**}	1.00	1.13	0.32	0.72	0.21	0.24	0.18	0.27	0.73	1.04^{***}
Wild oat biomass														
Total biomass (g) m^{-2}	666^{***}	468	480	449	374	309	188	187	110	73	–	–	364^{***}	297
Biomass (g) $plant^{-1}$ m^{-2}	42^{**}	29	32	30	31	26	24	23	27	18	–	–	31^{***}	25

Table 3
Yellow starthistle and wild oat replacement series means for combined data from 2006 and 2007. Values are given for the inoculated (*I*) and non-inoculated (*N*) plots at each proportion of YST to wild oat, and then combined for the main rust effect of all proportions. Statistical differences denoted as follows: $P < 0.10(*)$, $P < 0.05(**)$.

YST:wild oat proportions	0:100		25:75		50:50		75:25		100:0		Combined	
	I	*N*	*I*	*N*	*I*	*N*	*I*	*N*	*I*	*N*	*I*	*N*
YST biomass												
Total biomass (kg) m^{-2}	–	–	2.7	3.1	2.2	3.4^{**}	2.7	2.5	2.9	3.7	2.6	3.2^{**}
Biomass (kg) $plant^{-1}$ m^{-2}	–	–	0.72	0.78	0.30	0.51^{*}	0.24	0.23	0.21	0.26	0.36	0.44
Wild oat biomass												
Total biomass (g) m^{-2}	612	522	431	335	243	230	171	157	–	–	364^{*}	311
Biomass (g) $plant^{-1}$ m^{-2}	38	33	39	29	33	30	41	47	–	–	38	35

Fig. 4). Though not significant, there was a 50% increase in the 31–50 leaf class in inoculated plots compared to non-inoculated plots. These differences are likely due to the increased rate of leaf senescence on larger plants infested with *P. jaceae*, which resulted in a higher percentage of plants with lower leaf numbers.

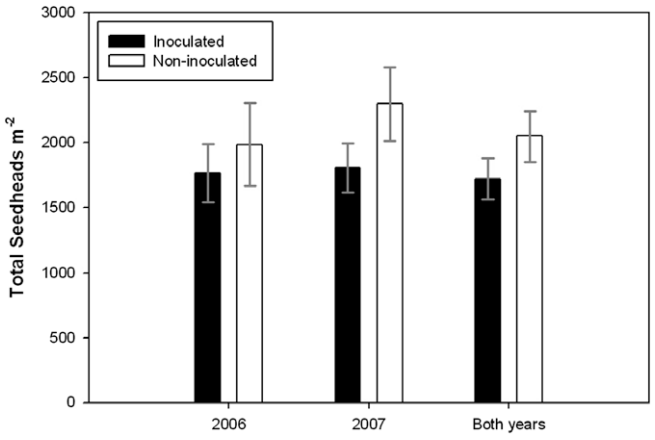

Fig. 3. Replacement series total seedhead means for all proportions combined in 2006, 2007, and both years combined (±SE). For both inoculated and non-inoculated $N = 16$ for 2006, $N = 20$ for 2007, and $N = 36$ for both years.

The pre-bolting diameter of YST rosettes was measured in the replacement series experiment in 2007. Corresponding to the

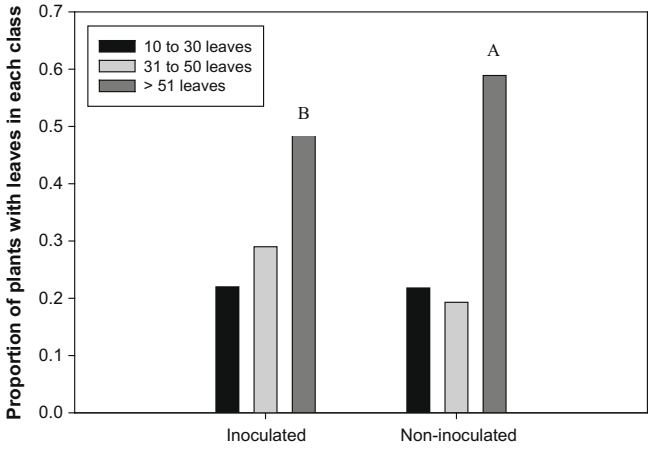

Fig. 4. Replacement series proportion of plants in each leaf count category between *Puccinia jaceae* inoculated and non-inoculated plots. Data are combined for 2006 and 2007. Leaf numbers are divided into three classes. The graph represents the proportion of plants in each leaf number class. Both inoculated and non-inoculated treatments were represented by 144 plants. Different letters represent significance at $P < 0.05$. Only plants in the >51 leaves category showed significant difference between inoculated and non-inoculated treatment.

Table 4
Competition indices for 2006, 2007, and combined years. Values are given for the inoculated (*I*) and non-inoculated (*N*) plots at each proportion of YST to wild oat, and then combined for the main rust effect of all proportions. Y and O are yellow starthistle and wild oat, respectively. Statistical differences denoted as follows: $P < 0.10(*)$ and $P < 0.05(**)$.

YST:wild oat	25:75		50:50		75:25		Combined	
	I	*N*	*I*	*N*	*I*	*N*	*I*	*N*
2006								
RCC[a]; Y–O	1.89	2.06	1.25	1.91	0.89	0.49	1.35	1.49
RCC; O–Y	2.23	0.63	1.22	4.72	1.32	2.61	1.59	2.65
RYT[b]	1.38	1.14	1.26	1.24	1.34	1.18	1.33	1.18
Aggressivity[c]	−0.42	−0.17	0.12	0.21	0.52	0.21	0.08	0.08
2007								
RCC; Y–O	8.15	5.64	3.63	3.37	1.81	1.47	6.64	5.05
RCC; O–Y	0.17	0.26	0.40	0.42	0.60	0.80	0.30	0.44**
RYT	2.10	1.87	1.24	1.48	1.08	0.82	1.64**	1.42
Aggressivity	0.86*	0.34	0.62	0.64	0.74	0.49	0.65**	0.33
Combined years								
RCC; Y–O	5.02	3.85	2.44	2.64	1.35	0.98	2.94	2.49
RCC; O–Y	1.20	0.45	0.81	2.57	0.96	1.70	0.99	1.57
RYT	1.74	1.50	1.25	1.36	1.21	1.00	1.40	1.29
Aggressivity	0.22	0.09	0.37	0.43	0.63	0.35	0.41	0.29

[a] Relative crowding coefficient according to Harper (1977). RCC values were taken for each block.
[b] Relative yield total according to Radosevich et al. (1997).
[c] Aggressivity according to Radosevich et al. (1997).

reduction in the number of plants with high leaf number following infection with *P. jaceae*, the combined data for all proportions showed a significant reduction (29%, $P < 0.05$) in the radius of rosettes inoculated with *P. jaceae*.

3.4.4. Competitive indices

Relative crowding coefficient (RCC) is a measure of the competitive ability of one species relative to another (Harper, 1977). Higher values indicate higher competitive strength (Firbank and Watkinson, 1985; Newman, 1983). A reduction in the RCC value when YST plants are infected with *P. jaceae* would indicate reduced competitive ability compared to non-inoculated plants. Combined data for each year (2006 and 2007) and for both years together showed no statistical differences for YST competitiveness between inoculated and non-inoculated plots (Table 4). However, overall RCC values differed by year; YST was more competitive with wild oat in 2007 compared to 2006. This corresponded to the higher biomass values in 2007 relative to those of 2006. Similarly, the RCC values for wild oat were not significantly different between the two treatments at any proportion; however, combined data in 2007 show wild oat in non-inoculated plots to have higher competitiveness with YST compared to inoculated plots, although both inoculated and non-inoculated treatments were far less competitive to YST in 2007 compared to 2006.

Relative yield total (RYT) is a measure of resource complementarity (de Wit and van den Bergh, 1965). RYT values close to 1 indicate that two species have similar demands on the same limiting resource(s). Values higher than one indicate that while species may compete for some of the same resources, they also utilize different resources. We detected differences in RYT only for the 2007 combined data (Table 4). The higher value occurred in inoculated plots, suggesting that *P. jaceae* infection caused YST to utilize a somewhat different resource set than wild oat. However, no significant differences in RYT were seen for individual proportions, for 2006 overall, or for the two years combined.

Aggressivity is also used to infer competitiveness between species (Radosevich et al., 1997). An increase in the competitiveness of a given species is reflected as an increase in relative yield. We calculated relative yield for each species at every proportion and used these values to determine the mean aggressivity index. We then used aggressivity values to compare the competitiveness of YST in the presence or absence of *P. jaceae*. YST aggressivity did not differ between inoculated and non-inoculated plots in 2006 or for both years combined. However, in 2007 the inoculated YST was actually more aggressive with wild oat compared to the non-inoculated plots (Table 2).

When relative yields of both species, inoculated and non-inoculated, were combined for the two years and plotted on a replacement series graph, there were no significant differences between the intersection points for either YST ($P = 0.2998$) or wild oat ($P = 0.836$) (Fig. 5). Thus, despite some differences between inoculated and non-inoculated plots in growth parameters, there was little overall effect of *P. jaceae* on the competitive interaction between YST and wild oat.

4. Discussion

Chlorophyll measurements can be used to estimate photosynthetic output in plants (Richardson et al., 2002; Markwell et al., 1995). Our results indicate that YST leaves with more than 15 pus-

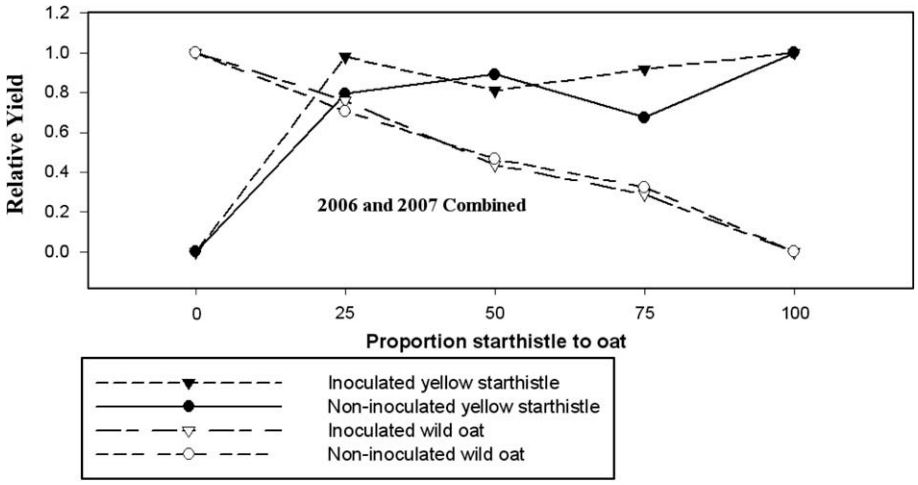

Fig. 5. Replacement series graph of relative yield of yellow starthistle and wild oat at each proportion. Data combined from 2006 and 2007. Relative yields for each species were compared using multi-response permutation procedures (MRPP). There were no significant differences between the intersection points for either YST ($P = 0.2998$) or wild oat ($P = 0.836$).

tules have decreased chlorophyll and, therefore, photosynthetic rates. This is most likely due to the loss of leaf area caused by the physical presence of the sporulating rust (Shtienberg, 1992). Paul and Ayres (1984) found that rust-infected groundsel (*Senecio vulgaris* L.) had both reduced size and reduced photosynthetic output compared to healthy plants. While the modest reductions of YST biomass in the replacement series experiments may be due to the effect of *P. jaceae* on photosynthetic output, a similar response was not seen in the YST monoculture density pots. *P. jaceae* infection reduced yellow starthistle photosynthetic output on a per leaf basis, but it is possible that uninfected leaves compensated for this effect. Paul and Ayres (1984) and Livne (1964) both showed that healthy leaves on the same infected plants had increased photosynthesis levels, thereby compensating to some degree for rust-induced decreases on infected leaves.

Increased *P. jaceae* severity can also result in increased leaf senescence in YST (Woods and Popescu, 2007). Rust-induced senescence has been reported in other plant species (Baudoin et al., 1993). A decline in the overall number of YST leaves, along with a decline in the photosynthetic area of infected leaves, were the most likely contributors to the modest declines in YST plant biomass.

The responses of YST to *P. jaceae* infection were very different in 2006 and 2007. In 2006, *P. jaceae* had no significant effect on YST biomass, leaf number, reproductive output, or competitive indices, though there were trends towards reductions in biomass and seedhead numbers. However, total biomass m^{-2}, biomass $plant^{-1}$, leaf number, and rosette radii were larger in the non-inoculated YST in 2007, and although not statistically significant, total seedhead numbers were also greater in the non-inoculated plots. These negative effects of *P. jaceae* on YST in 2007 corresponded with a positive response in wild oat, seen as an increase in biomass m^{-2} and biomass $plant^{-1}$.

In both years, field sites received supplemental irrigation, 50 mm in February 2006 and 90 mm between December and March 2007. However, in 2006, there was substantially more rain (534 mm) during the rosette stage (mid-Dec to mid-April) compared to 2007 (175 mm), and the field site was flooded for a portion of March in 2006. Even with the addition of 90 mm of irrigated water in 2007, the total precipitation for the year was 150 mm (31%) below normal. Thus, it is possible that the different response to *P. jaceae* seen in the two years was due to precipitation patterns. Interestingly, YST biomass m^{-2} was 54% and 17% greater in the non-inoculated and inoculated plots, respectively, in the drought year of 2007 compared to 2006. Although Shishkoff and Bruckart (1996) did not find a significant response difference in YST infected with *P. jaceae* under greenhouse drought conditions, another study found differences in bean rust sporulation under drought conditions (Duniway and Durbin, 1971).

There were no differences among any growth parameter in the inoculated and non-inoculated YST monocultures either within or across years. In contrast, our results indicate that *P. jaceae* has more of an effect on YST under conditions of interspecific competition. Similar results were reported for velvetleaf (*Abutilon theophrasti* Medik.) infected with the fungal pathogen *Colletotrichum coccodes* (Wallr.) S. Hughes (Barney et al., 2006; DiTommaso et al., 1996). In these studies, the pathogen gave a greater reduction in growth when velvetleaf was grown in competition with soybean (*Glycine max* (L.) Merr.), compared to monocultures of the weed. Wild oat, which elongates early in the season, may have shaded YST plants, thus enhancing the effect of infection in reducing leaf number and rosette size. Shading has been shown to be one of the most important factors limiting the growth of YST (DiTomaso et al., 2003; Roche et al., 1994). A similar situation would not occur in the monoculture plots since all YST plants would be in the rosette stage until late April, with no other vegetation occupying the canopy layer.

While these results provide some evidence that *P. jaceae*-infected YST has lower biomass and other parameters when growing in competition with annual grasses, our results did not show that such a response translated into a significant reduction in the competitive ability of YST (Table 2). Furthermore, it has been shown that any long-term management of YST depends upon a significant reduction in the soil seedbank (DiTomaso et al., 2006a, 2006b, 1999a). Though *P. jaceae* did reduce YST biomass under interspecific competition, this did not lead to a significant reduction in reproductive output. The largest reduction in seedhead output we observed was 21% in 2007. Maddox (1981) found that YST plants produce an average of 104 seedheads per plant, each seedhead producing an average of 38–83 seeds (Benefield et al., 2001; Maddox, 1981). Given that a YST population can produce 3000–10,000 seeds per m^2 (DiTomaso et al., 1999a), a 21% seedhead reduction is unlikely to have a significant effect on YST density.

5. Conclusion

Puccinia jaceae reduced photosynthetic output on a per leaf basis and produced a modest decrease in plant biomass under conditions of interspecific competition with a common annual grass. However, in the two years of this study, *P. jaceae* did not cause a major decline in seedhead production. Furthermore, the biomass reduction in the replacement series experiment was not enough to lower the overall competitive advantage of YST over wild oat. Therefore, we conclude that *P. jaceae* alone will not significantly control *C. solstitialis* and is unlikely to lead to dramatic population reductions of this invasive species in the western United States. However, because it generally requires multiple years for the effects of a biocontrol agent to become fully apparent (Woods et al., 2007; Harris, 1985), long-term monitoring may provide more insights into the effects of *P. jaceae* on YST.

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