Establishment of the Yellow Starthistle Rust in California: Release, Recovery, and Spread

Dale M. Woods, California Department of Food and Agriculture, Sacramento 95832; Alison J. Fisher, Exotic and Invasive Weeds Research Unit, United States Department of Agriculture–Agricultural Research Service, Albany, CA; and Baldo Villegas, California Department of Food and Agriculture, Sacramento

ABSTRACT

The rust fungus *Puccinia jaceae* var. *solstitialis* is the first pathogen released for biological control of yellow starthistle (*Centaurea solstitialis*). From 2004 to 2006, the pathogen was released at 176 sites in 40 counties throughout the state of California. Release sites were evaluated 1 to 3 months after inoculation, depending on the year. After 1, 2, and 3 years, the percentages declined to 19 to 21, 9 to 10, and 3% respectively. Spread was detected at 19% of the sites with rust infection, with an average distance of 21 m (±13.3 standard error). The greatest spread occurred at a site in Sonoma County. At this site, the rust spread to over 37 acres 1 year after it was released and has remained in this area for three seasons. Re-emergence 1 and 2 years after inoculations was more likely in Northern (above 40°N) compared with Southern California (below 36°N). In general, re-emergence was more likely at lower elevations when release sites were within 150 km of the coast. Overall, the rust has not demonstrated a strong record of persistence based on these observations.

Due to the scale of the YST infestation in California, a large quantity of *P. jaceae* var. *solstitialis* spores were needed for the state-wide release program. However, greenhouse propagation of the rust for distribution is labor intensive (26). In order to use available spores more efficiently in the future, more information is needed regarding where the rust is likely to establish. To better understand this process, sites were monitored for reemergence and spread after releases. The objectives of this study were to determine where inoculations result in infected plants, determine where multiyear reemergence occurs, document disease spread, and identify regional characteristics where the rust is most likely to be successful.

MATERIALS AND METHODS
Fungal isolate and inoculum source. *P. jaceae* var. *solstitialis* field isolate FDWSRU 84-71 was collected near Sivas, Turkey in 1984 (3). Following quarantine evaluation, this isolate was moved under permit from the Foreign Disease Weed Science Research Unit in Ft. Detrick, MD, to the California Department of Food and Agriculture (CDFA), Sacramento, CA to establish a rust rearing program. Uredinospores produced on inoculated YST rosettes in a greenhouse were vacuum harvested and stored at −70°C for future field releases (26).

Distribution program. In 2004, 2005, and 2006, the CDFA contacted the Agriculture Commissioner office in each county of California to invite participation in upcoming field releases of *P. jaceae*. Interested counties sent at least one representative to a series of training sessions. Participants received classroom training on rust biology and were given directions for releases and monitoring. County representatives were provided with an “inoculation kit” to perform the release. Each kit contained a gelatin capsule containing urediniospores of *P. jaceae* (200 mg in 2004 and 100 mg in 2005 and 2006), a small vial of Tween 20 (polyoxyethylene sorbitan mono-laureate), cheesecloth, a 250-mL household finger-pump spray bottle, and a dew tent. The dew tent was a 1-m³ PVC frame with 20-cm legs, covered by 10-mil black plastic sheeting glued to the 1-m² frame with a 30-cm overlap of sheeting on each side. The dew tent was intended to maintain heat and humidity on freshly inoculated...
plants, increasing the probability of infection (3,13). All releases were performed under annual pesticide research authorizations obtained through the California Environmental Protection Agency. In total, 29 inoculations occurred in 20 counties between 4 March and 5 June 2004, 75 inoculations in 38 counties between 7 March and 6 June 2005, and 72 inoculations in 32 counties between 23 March and 29 August 2006. A map of release sites is included in Figure 1.

**Inoculation procedures.** Most sites were selected by county or other local personnel. Biologists were asked to select highly protected, preferably moist sites in 2004 and 2005 but were allowed to use their own discretion with site choice in 2006. At each site, a 1-m^2^ plot with YST rosettes as the predominant vegetation was chosen. Plots were expected to be part of a larger YST stand and representative of local infestations.

The corners of each release plot were marked by wooden stakes and tall, dead vegetation that might puncture the dew tent was removed. The entire urediniospore sample was added to a 0.5-liter plastic bottle containing 200 ml of water and 5 to 6 drops Tween 20. The mixture was intermittently swirled for 20 min so the spores could enter the suspension. The suspension was then filtered through four layers of cheesecloth into a spray bottle and the entire solution was sprayed onto the 1-m^2^ plot. After pustules were detected, monitoring switched to a 2- to 3-week schedule to evaluate in-season persistence and to measure rust spread from the original inoculation site. Because previous work indicated that the latent period was approximately 3 weeks in the spring (3,14,26), pustules developing outside the tent 2 to 4 weeks post inoculation were considered to result from drift during inoculations, whereas pustules developing outside the tented area after that time were considered to be the result of spread. Spread was determined as the greatest distance from the inoculation plot detected during a 30-min visual survey. Monitoring ceased when the rust disappeared or when plants reached maturity and leaves were no longer green.

In order to evaluate the multiyear persistence and spread of the rust, most sites were revisited annually each spring to look for signs of infection. Although yearly visits were encouraged, not all sites were visited each year. Spread at one site was substantial 16 months post inoculation; therefore, the entire infestation of YST was surveyed. The leading edge of the infestation was ground surveyed, GPS readings were collected, and extent of spread calculated.

A final statewide survey was completed in 2007 to assess the long-term persistence of the rust at each release site. From March to July 2007 the authors of this study attempted to follow up on all release sites. Data on rust reemergence was collected for either 1, 2, or 3 years, depending on how long the site had been in existence. Each site was surveyed for 30 min and rust was noted as present or absent in the general area of the original release plot. If the rust was present, the greatest distance spread from the release plot was measured. In the area of highest rust concentration, the number of plants infected out of 100 plants was recorded. Means and standard errors were calculated using Microsoft Excel. Excel was also used to determine if there was a correlation between disease incidence in 2007 and plant density measured at the time of inoculation.

**Comparison of regional characteristics.** To determine regional characteristics associated with reemergence of the rust after a dormant season, each site was categorized by Sunset climate zone (5), California floristic province region (15), elevation (0 to 152, 152 to 457, and >457 meters), distance to the ocean (to 75, 75 to 150, and >150 km), and latitude (32 to 36, 36 to 38, 38 to 40, and 40 to 42°N). For each reemergence category, a dependent variable was assigned: 0 if no rust was present 1 to 3 months after inoculations, 1 if the rust was present 1 to 3 months after inoculations, 2 if the rust was present 1 year after inoculations, and 3 if the rust was present 2 years or more after inoculations.

Statistical analyses comparing regional characteristics were carried out using SAS Institute software (version 9.1). We used the Proc Glimmix procedure to determine the effects of elevation, distance to the ocean, latitude, and their interaction on reemergence. The distribution was multinomial and contrasts were used to determine the direction of each significant effect. Sunset zone and California floristic province could not be included in the Glimmix analyses due to empty cells. Instead, the χ² test was used to determine the effect of Sunset zone and California floristic province on reemergence. Data from sites in Sunset climate zones 20 to 21, 22 to 24, and 18 to 19 (11 sites total) and floristic provinces South West, Modoc

---

![Fig. 1. Map of *Puccinia jaceae* release and reemergence sites in California from 2004 to 2006.](image-url)
Plateau, and Cascade Range (27 sites total) were removed from the χ² test because there were too few sites in those regions.

RESULTS

Inoculation success. Infection was successful at all but 2 of the 29 sites inoculated in 2004 (Table 1). Severe drought killed the YST within a few days of inoculation at one site and the failure of infection was unexplained at the other site. Although infection at the 75 release sites in 2005 was generally successful, no obvious reason was noted for the slightly increased failure rate over the previous year. In all, 13 of the 72 release sites in 2006 were not checked for 12 months after the releases; therefore, inoculation success is unknown for these sites. However, pustules were found at 41 of the remaining 59 sites. As our requirements for inoculation protocol became more lenient over time and the number of sites inoculated increased, the percentage of sites where inoculations resulted in no obvious infection increased from 7% in 2004 to 13% in 2005 and 31% in 2006. The mean disease incidence across all years was 53.7% (+3.5 standard error [SE], n = 90). There was no relationship between plant density in the release plot (minimum = 13, maximum = 1,672 plants m⁻²) and disease incidence. The associated regression equation was \( y = 0.006x + 51.7, r^2 = 0.003 \), where \( x = \) plant density and \( y = \) disease incidence.

Latent period. Pustules were noticed in the field as early as 14 days post inoculation. Latent period was estimated at 17 sites in 2004, 26 sites in 2005, and 20 sites in 2006. The average duration for pustule emergence was similar in 2004, 2005, and 2006, with 22.2 (+1.4 SE), 22.4 (+0.9 SE), and 19.7 (+0.9 SE) days respectively.

Rust persistence following inoculations. The rust did not persist well during the first year at the majority of our sites. Secondary cycling, production of a second generation of uredia during the growing season, was seldom apparent. Sixty-two sites that were successfully inoculated were repeatedly evaluated during their first year post inoculation. Nine of the sites were either destroyed or not evaluated the following year. The remaining 53 sites were divided into two categories: those where the rust reemerged 1 year after inoculation and those where the rust did not reemerge. Sites in these two categories were then compared for the history of rust persistence during the first summer following inoculation. Rust pustules were detected an average of 81.6 days post inoculation (+11.2 SE, \( n = 13 \)) on sites where the rust reemerged the following year. In contrast, at sites where the rust did not reemerge the following year, rust pustules were detected an average of 56.3 days post inoculation (+4.4 SE, \( n = 40 \)).

Multiyear reemergence. The percentage of sites with rust reemerging a full year after release was very low (18 to 27%) but fairly consistent over all years (Table 1). Reemergence continued to decline each additional year (Fig. 1; Table 1). Eleven sites were not visited in 2007 due to time constraints or the remoteness of the site. Of these 11 sites, only 1 had any indication of rust in a previous visit. Of the 150 remaining sites that were visited in 2007, only 21 had rust-infected YST plants (Table 1).

We were not able to evaluate reemergence at all sites because of the unanticipated destruction of several of the release sites. In total, 14 of the 176 sites (8%) had been destroyed within a year after the fungus had been released. Additional losses occurred in succeeding years such that, overall, 16% of the sites were destroyed within 2 years and 28% were destroyed within 3 years. The most common factors involved in site destruction were herbicide use (intentional and accidental), flooding, wildfires, repeated mowing, and construction projects.

Disease incidence was determined for 17 sites in 2007. The majority (10) of these sites had been inoculated the previous year and spread was limited to the immediate area. The mean incidence was 8% of the plants infected (+2.1 SE, maximum = 35%).

Spread. Rust pustules were often found outside the plots on plants within 1.5 m of the inoculation less than one latent period after the first pustules emerged within the plot, suggesting that short-range drift was common. Pustules were detected beyond 2 m within the first 3 months after a release on 25 occasions (or 19% of the positive releases), with an average distance of 5.44 m (+0.8 SE) and a maximum of 15 m. An additional 14 sites that did not have any apparent spread the first season developed pustules outside the plots in subsequent years, bringing the total number of sites with spread to some point to 39 (29% of the positive releases). The average maximum multiyear spread was 21 m (+9.3 SE). In spite of exhibiting spread in previous years, 82% of these sites eventually failed and had no evidence of rust in 2007. At the four sites with spread over 40 m, the rust was still present in 2007 (Fig. 2).

Substantial multiyear spread occurred in three locations. One location is dry rangeland in Yolo County (Madison), where a 2005 inoculation spread 220 m by May 2007. An oak woodlands site in Santa Clara County, a 2005 release spread 39 m by 2007. The farthest spread resulted from a 2004 release in Sonoma County. By August 2005, the rust had spread to over 37 acres and YST has remained highly infected for three seasons.

Effect of regional characteristics. Elevation (\( P = 0.0186 \)), latitude (\( P = 0.0143 \)), and the interaction between elevation and distance to the ocean (\( P < 0.0119 \)) each impacted reemergence 1 year after inoculations. Reemergence was more likely above 40°N (\( \chi^2 = 4.2, df = 1, P = 0.0405 \)), between 38 and 40°N (\( \chi^2 = 5.97, df = 1, P = 0.0145 \)), and between 36 and 38°N (\( \chi^2 = 4.89, df = 1, P = 0.0270 \)), compared with sites below 36°N. When sites were within 75 km of the coast, reemergence was greater below 152 m compared with sites above 457 m (\( P = 0.0208 \)). When sites were between 75 and 150 km from the coast, reemergence was greater below 152 m compared with sites between 152 and 457 m (\( P = 0.0023 \)) but reemergence was lower at sites below 152 m compared with sites above 457 m (\( P = 0.0008 \)).

For sites that were evaluated 2 years after inoculations, elevation (\( P = 0.2205 \)) and distance from the ocean (\( P = 0.2900 \)) had no effect on reemergence. Reemergence after 2 years was more likely above 40°N compared with sites between 38 and 40°N (\( \chi^2 = 4.83, df = 1, P = 0.0280 \)) and sites below 36°N (\( \chi^2 = 4.93, df = 1, P = 0.0264 \)).

When sites were evaluated 1 year after releases, there was no effect of floristic region (\( \chi^2 = 8.4, df = 6, P = 0.2066 \)) or Sunset climate zone (\( \chi^2 = 11.6, df = 6, P = 0.0692 \)).

DISCUSSION

The release of the nonindigenous fungus \( P. jaceae \) was highly anticipated by weed control specialists in the western United States. This was the first plant pathogen to be released in the 48 states as a biological control agent under the modern permitting system. A substantial prerelease effort was required to adequately support the permitting process. Successful greenhouse production of rust spores (26) enabled a multiyear statewide release program. Post-release monitoring of establishment and spread of the YST rust was essential to justify this commitment of resources, and the resulting data are essential for developing new protocols when pathogens are released for weed biological control in the future.

Table 1. Detection of \( P. jaceae \) at various times post release

<table>
<thead>
<tr>
<th>Year released</th>
<th>No. of sites</th>
<th>Percent sites with rust at the time monitored (numbers of sites)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 to 3 months</td>
<td>12 to 14 months</td>
</tr>
<tr>
<td>2004</td>
<td>29</td>
<td>93 (27/29)</td>
</tr>
<tr>
<td>2005</td>
<td>75</td>
<td>87 (65/75)</td>
</tr>
<tr>
<td>2006</td>
<td>72</td>
<td>69 (41/59)</td>
</tr>
</tbody>
</table>

* Data are percentage of sites (raw data) with \( P. jaceae \). Includes only data from sites where yellow starthistle was still present at inspection.
Infection after inoculations was generally successful statewide. This was not unexpected because previous studies (26) had demonstrated a uniform susceptibility among 62 accessions of YST, and temperature and dew requirements for infection are well established (3,14). It is not clear why infection following inoculations dropped from 93% in 2004 to 69% in 2006. One explanation may be that many of the 2006 sites were inoculated later in the year than the previous year’s trials. In 2004 and 2005, most cooperators released *P. jaceae* in March and early April. In 2006, many sites were inoculated after mid-April, including all of the sites that failed to get infected. Reduced dew duration as the spring progresses is a possible cause for infection failure. The utilization of dew tents maintained dew-like conditions for as long as 16 h but, upon their removal, continued leaf wetness was affected by atmospheric conditions. In controlled laboratory studies at 20°C, 16 h of dew is sufficient for infection (3). Potentially, the cooler night temperatures in the field require a longer dew period. Nonoptimal temperature and dew duration were similarly thought to have caused mid- and late-season failures with the Dyers woad rust, *P. thlaspeos*, which only produced pustules after spring and not summer or fall inoculations (16).

Latent period data reported here was not substantially different from the results of a previous study (14) and only slightly longer than greenhouse and growth-chamber experiments (3,26). Bennett et al. (3) determined that the latent period was prolonged (15 days) at lower constant temperatures (15°C). Field temperature was not measured in our study but night temperatures are often lower than 15°C during the winter in California; therefore, it is not surprising that it took longer (19 to 22 days) for pustules to emerge.

Although *P. jaceae* can successfully be inoculated on YST in the field, it does not seem to be able to persist through the summer and reemerge in succeeding years at very many locations. Because host susceptibility is not a likely explanation (26), environmental factors most likely limit long-term success. Continued secondary cycling seems important to support reemergence the following year. In this study, urediniospores were present a month longer (81.6 versus 56.3 days after inoculation) at sites where the rust reemerged the next season. Rain events which are common in the winter in California decline markedly in April and May and, therefore, secondary infections, caused by urediniospores, must rely on natural dew development. As day length increases, dew duration decreases in the field. Dew duration is shorter in the early summer compared with the spring and, therefore, the likelihood of reinfection declines.

Teliospore production is essential for overwintering because urediniospore survival in the field is negligible 3 weeks after production (14). As YST matures, urediniospore production declines and teliospores become the predominant spore stage on the plant, comprising up to 100% of the spores by mid-August (11). With limited cycling of urediniospores, the potential for teliospore production declines. Additionally, leaves that are the most infected tend to have the shortest lifespan (24,29,30) and, thus, the potential for teliospore production is further reduced in severe infections. It is possible that, in most locations, there was insufficient teliospore production for overwintering and production of basidiospores the following spring. Therefore, early-season leaf loss following inoculation, accompanied by marginal conditions for teliospore production, will lead to limited reemergence opportunities in succeeding years and, possibly, disease disappearance locally. Other rusts that have been successful as weed biological controls have either been on perennial woody plants (for example, *Uromycladium tepperianum* on the invasive tree *Acacia saligna*; 18) or are systemic (for example, *P. thlaspeos* on dyer’s woad; 16).

An introduced biological control agent is usually considered to have established if it persists for two full seasons. As such, *P. jaceae* could be referred to as established in California but only poorly so. With the progressively declining level of reemergence in most sites, 2 years may be premature to declare establishment with this particular rust species.

The spread of this rust from release sites has also been disappointing. The relatively short-distance spread observed confirms that the rust can function under California conditions but is not likely to meet the goals for an effective classical biological control agent. Other biological control rusts have demonstrated far superior long-distance spread. Chondrilla rust, caused by *P. chondrillina*, spread 3.4 km in 1 year in California (10) and 320 km in a 9-month period following release in Australia (1,8). Musk thistle rust (*P. carduorum*) released in 1987 in Virginia had spread as far as Missouri by 1994 (2) and as far as California by 1998 (28). Dyers woad rust, with only one generation per year, spread 300 km in a little over 20 years (16). The sub-
stantial spread of *P. jaceae* at the Sonoma site bordering the San Pablo Bay suggests that the rust is well adapted to coastal conditions. However, this region of the state is not the area most impacted by YST. The greatest acreage covered by YST occurs in areas with large weed infestations in the central part of the state.

The analyses of regional characteristics such as distance to ocean, latitude, elevation, and plant or climate zone did not result in definitive predictions for where to release the rust in the future. Most sites where the rust reemerged were in and around the San Francisco Bay Area but there were many sites in this region where the rust did not reemerge. Based on the slightly greater successes in establishment and spread, future release programs should be focused in areas in Northern (above 36°N) California, at lower elevations, and within 150 km of the coast. YST is present in coastal Northern California; therefore, it should be possible find additional populations meeting these criteria.

Establishing a large-scale distribution program for a rust biological control agent is a complicated process. Production and storage of inoculum were early challenges that have been addressed (26). Challenges encountered while conducting research reported here were often due to the extensive distances between release sites and the number of people involved in the release program. For example, it was difficult to distibute spores to practitioners fast enough so that viability was not compromised. It was impractical to insist that practitioners visit sites for biweekly follow-up and it was difficult to avoid the loss of release sites due to unforeseen circumstances. For example, one site failed because an unusually large infestation of spider mites consumed all developing pustules (D. Woods, personal observation). In addition, record keeping became substantially less complete as the novelty of the project had waned. As a result, a lower percentage of sites provided data useful for latent period calculation, reemergence, and spread as time progressed. Additionally, many of these sites were remote and could only be visited infrequently.

In order to be an effective biological control, the rust would have to maintain a sustained disease severity throughout the season and repeatedly reemerge. Overall, the rust has not demonstrated a strong record of persistence except for few select sites. Although *P. jaceae* is not likely to reach epidemic levels on YST in the interior of California, the results of this study show that estuarine sites, similar to the one in Sonoma County, can support *P. jaceae* populations. Sites with reoccurring *P. jaceae* populations will continue to be monitored so that we may determine if infection by the rust results in measurable control. If the rust has any impact, it will be geographically limited. Region-wide control of YST will have to be based on other components of an integrated pest management program.

**ACKNOWLEDGMENTS**

We thank the staff of the county agriculture commissioners for their efforts in securing sites, performing inoculations, and continued monitoring; L. Whitehand for statistical consulting; and A. Yrie for production of the maps.

**LITERATURE CITED**


