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Developing an optimal release strategy for the rust fungus *Puccinia jaceae* var. *solstitialis* for biological control of *Centaurea solstitialis* (yellow starthistle)

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Abstract

The rust fungus *Puccinia jaceae* var. *solstitialis* (*P. j. solstitialis*) was first approved for release in California in 2003 as a classical biological control agent for *Centaurea solstitialis* (yellow starthistle, Asteraceae). It is difficult to produce large quantities of this obligate pathogen so it was necessary to develop an optimal release strategy for the efficient use of urediniospores. In 2005–2006 field experiments were conducted in two distinct habitats types, the coastal hills and Central Valley, CA, to determine the optimal month for introductions, and to determine if enclosing plots in tents at the time of inoculation was necessary to achieve high levels of infection. All releases resulted in infected plants at both sites for both years. At the Central Valley site near Woodland CA, disease incidence was higher than at the coastal hills, tent enclosures had no effect on infection, and the pathogen persisted throughout the growing season. One year after the 2005 release, *P. j. solstitialis* had reappeared in most Central Valley plots, although early season releases in 2005 resulted in greater severity in 2006 than the late season releases. In the coastal hills near Napa, CA, tent enclosures improved disease incidence and severity after January and May releases, perhaps by retaining moisture, but the pathogen did not persist in all plots until plant senescence, and there was no reinfection (the following spring) at this site. The rust fungus did not have a negative affect on plant mortality, biomass, or flower production at either location. Our results show that infection can be achieved from January to June, and tent enclosures sometimes appeared to increase infection; however, reinfection is probably limited by local environmental conditions.
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1. Introduction

Yellow starthistle (YST, *Centaurea solstitialis* L., Asteraceae) is a noxious invasive weed infesting over 7 million ha of California (CA) rangelands and natural areas (Pitcairn et al., 2006) and over 8 million ha of the western United States (US) and Canada (Skinner et al., 2000; Duncan, 2001). Yellow starthistle displaces desirable plants, lowers the value of recreational lands, and the spiny flowers deter feeding by grazing animals (Sheley et al., 1999).

Yellow starthistle is a winter annual adapted to the mild wet winters and dry summers of the Mediterranean (Maddox, 1981). Seeds usually germinate soon after the beginning of fall rains, rosettes develop slowly during the winter, plants bolt in late spring and flower continuously until the plant senesces from lack of moisture. Six exotic insect species have been introduced for biological control of YST, all of which attack flower heads and destroy developing seeds (Turner et al., 1995). However, these have not generally reduced populations to acceptable levels (Balciunas and Villegas, 1999; Pitcairn et al., 2002).

The rust fungus, *Puccinia jaceae* Otth var. *solstitialis* (*P. j. solstitialis*) was first released for biological control of

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YST in 2003 (Woods and Villegas, 2004). It is a macrocyclic, autoecious rust, native to areas of Eurasia with a Mediterranean climate, from Spain to Turkey (Savile, 1970). Since its limited introduction to North America, multiple cycles of urediniospore production and infection have occurred on YST at some sites throughout the spring and early summer, followed by teliospore production and ultimately, overwintering on senesced plant material (A. Fisher and D. Woods, personal observation). After YST seedlings germinate during the wet season (which begins in late fall), teliospores most likely produce basidiospores, which infect plants and produce pycnia, and then aecia in the early spring (Savile, 1970). Pycnia have been observed on YST in late February in California (Fisher et al., 2006).

The host range of *P. j. solstitialis* is limited to plant species in the Asteraceae, Tribe Cardueae, subtribe Centaureinae, particularly *C. solstitialis*, *C. cyanus* L. (bachelor's button) and *Carthamus tinctorius* L. (safflower) when tested in a quarantine greenhouse. Subsequent risk assessment data suggest that none of the non-target plants would be at risk following a field release in the United States (Bruckart, 1989, 2006). Studies to measure potential impact of *P. j. solstitialis* were conducted in quarantine. Repeated inoculations of 4-week-old YST plants, over a 3- to 4-week period, resulted in a 40% reduction in root weights and a 50% reduction in shoot weights (Bennett et al., 1991; Shishkoff and Bruckart, 1993). The negative effect on root biomass was even greater when plants were drought stressed (Shishkoff and Bruckart, 1996). Rust infection also reduced leaf lifespan and the total number of rosette leaves per plant (Shishkoff and Bruckart, 1996).

Puccinia jaceae var. *solstitialis* is the first pathogen to be approved for release for the classical biological control of a weed in the continental USA under permitting processes most recently required by the Animal and Plant Health Inspection Service (APHIS). A limited release of *P. j. solstitialis* occurred in April 2003, followed by releases in 20 counties in California in 2004 and 21 counties in 2005 (Woods and Villegas, 2005, 2006). During these years, 1-m^2 YST plots were inoculated once with an aqueous spore suspension in either March or April and covered with plastic tents overnight to maintain high humidity (D. Woods, unpublished data).

Due to the limited number of fungal pathogens used in classical biological control, there is little information regarding optimal strategies for releasing rust fungi to control terrestrial weeds (Rosskopf et al., 1999). Optimal greenhouse temperatures for *P. j. solstitialis* infection are between 15 and 20 °C with dew periods between 8 and 16 h (Bennett et al., 1991). It is not known whether we can achieve sufficiently high levels of infection to impact the weed under field conditions, which may be suboptimal, or how to improve infection. Methods that may increase leaf wetness after field inoculation include spore carrier formulation (Weidemann and Templeton, 1988; Quimby et al., 1989), plastic tents (Batchelor et al., 2004), and timing of applications to coincide with precipitation (Royle and Butler, 1987). However, sometimes these approaches are not necessary for the application of a pathogen for classical biological control. For example, *Puccinia chondrillina* Bubak & Syd., released for control of skeleton weed (*Chondrilla juncea* L.), became established in the western United States after dusting spores or applying spore suspensions in water (Emge et al., 1981). This agent successfully reduced skeleton weed populations in California (Supkoff et al., 1988). Similarly, the rust fungus *Maravalia cryptostegiae* Cummins was released successfully into inaccessible areas in Australia to control rubber-vine weed (*Cryptostegia grandiflora* R. Br.) using a water-based suspension dropped out of light aircraft (Tomley and Evans, 2004).

The purpose of this study was to (1) determine if there is an optimal combination of climate (i.e., temperature and moisture) and plant phenological stage to inoculate plants that results in a high rate of disease and reinfection, (2) determine if plastic tents improve initial infection and reinfection, and (3) determine if timing of inoculation affects mortality, plant biomass and flower head production of YST.

2. Materials and methods

2.1. Fungal isolate and inoculum source

Puccinia jaceae var. *solstitialis* field isolate FDWSRU 84–71 was collected by S.S. Rosenthal in 1984 east of Yarhisar and Hafik (near Sivas), Turkey. From 1984 to 2003 this field isolate was evaluated in quarantine at the USDA, ARS, Foreign Disease-Weed Science Research Unit, at Ft. Detrick, MD. Urediniospores for the present study were produced on 4- to 6-week-old potted YST plants inoculated with 50 mg of isolate FDWSRU 84–71 suspended in water with 0.15% Tween® 20 (polyoxyethylene sorbitan monolaurate; EM Science, Gibbstown, NJ). Inoculated plants were placed in a dew chamber at 20 °C for 16 h, and then held in a greenhouse for a minimum of 14 days until pustules formed. Spores were harvested using a vacuum spore collector and stored at −70 °C. Increases of inoculum took place at the California Department of Food and Agriculture, Sacramento, CA.

2.2. Site characteristics

Permanent experimental plots were established in January 2005 and 2006 at two sites: Napa and Woodland. The Napa site is an ungrazed rangeland valley in the coastal hills near Napa, Napa County, 427 m elev, dominated by YST, European annual grasses, native grasses and shrubs, and surrounded by oak woodland. It is part of the Inner North Coast Range California Floristic Province, Jepson climate zone 14 (Hickman, 1993). The Woodland site is ungrazed rolling hills rangeland, near Woodland, Yolo County, 53 m elev, in the Central Valley. It is dominated by European annual grasses and herbs, surrounded by