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# The effect of *Puccinia jaceae* var. *solstitialis* on the yellow starthistle biological control insects *Eustenopus villosus* and *Chaetorellia succinea*

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ABSTRACT

Six insect biocontrol agents have been introduced, with limited success, for managing the invasive plant yellow starthistle (*Centaurea solstitialis*). In 2003, a recently introduced fungal rust agent, *Puccinia jaceae* var. *solstitialis*, was approved for release in California. The presence of the rust in this multi-species complex was evaluated for its effect on performance of the two most common insect biocontrol agents for yellow starthistle, *Chaetorellia succinea* and *Eustenopus villosus*. To accomplish this, yellow starthistle was planted in 1 $m^2$ plots in monocultures at three densities and in a competition replacement series with wild oat (*Avena fatua*) in both 2006 and 2007. Twenty seedheads were dissected from each plot to evaluate the effect of *P. jaceae* on seedhead maturation and insect attack rate. In the replacement series experiment in 2007 and in 2006 and 2007 combined, *P. jaceae* caused a 35% and 20% increase, respectively, in the proportion of mature seedheads compared to total seedheads. However, there were no significant differences in the density experiment in either year or in the replacement series experiment in 2006. Although *P. jaceae* appeared to have a slight effect on yellow starthistle seedhead maturation, there was no effect of the rust on seedhead attack rates of either insect biological control, regardless of the experiment or year. These results indicate that *P. jaceae* does not interact significantly with the insect biological control agents for yellow starthistle.

## 1. Introduction

Yellow starthistle (*Centaurea solstitialis* L.) (YST), in the Asteraceae, is estimated to infest nearly 6 million hectares in California, Oregon, Washington, and Idaho (Duncan et al., 2004; Pitcairn et al., 2006). There have been multiple attempts to control the weed using biological control since the release of the first insect agent in 1969 (Pitcairn et al., 2004). Currently, six insect agents have been officially released (Balciunas and Villegas, 1999; Pitcairn et al., 2004), along with one accidental introduction (Balciunas and Villegas, 2001); all of these attack seedheads. Of these seven agents, only the accidentally introduced false peacock fly (*Chaetorellia succinea* Hering, CHSU) and the hairy weevil (*Eustenopus villosus* Boheman, EUVI) are considered widely distributed and potentially effective (see DiTomaso et al., 2006 for review; Pitcairn et al., 1999). A recently introduced biological control pathogen, *Puccinia jaceae* Otth var. *solstitialis* (yellow starthistle rust), was approved for release in 2003 (Woods et al., 2004, 2009) and has since been distributed state-wide (Woods et al., 2009). While greenhouse studies have shown that *P. jaceae* can increase leaf senescence and reduce YST shoot and root biomass (Bennett et al., 1991; Shishkoff and Bruckart, 1993), our field studies showed only minor effects on plant biomass, leaf number, and rosette diameter, and no significant effect on seedhead production (Woods and Popescu, 2007; O'Brien, 2008; O'Brien et al., 2010).

There has been substantial research into the effects and benefits of multiple biological control agents to control weeds (Harris, 1979; Myers, 1985; Denoth et al., 2002; Chandramohan and Charudattan, 2003). In an analysis of the biological control program for 50 plant species, Harris (1979, 1985) reported that, on average, four biological control agents had been released per target plant. The goal of these multiple-agent releases was to expose the target species to cumulative stress. However, Myers (1985) reported that only 30% of biological control projects were actually successful. She noted that among the successful biological control programs, a single agent was responsible 81% of the time. Thus, the multiple releases of biological controls does not generally increase the cumulative stress on the target species, but rather increases the probability that one of the agents will be successful, i.e., a 'lottery ticket' approach.

In recent years, there have been many studies focused on the interactions of multiple biological control agents as they affect each other and the host plant (Mihail et al., 1998; Kluth et al.,

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2001; Peacock et al., 2003; Crowe and Bourchier, 2006; Seastedt et al., 2007). For example, a number of studies have shown an antagonistic relationship between different biological control agents on a host plant (Kruess, 2002; Peacock et al., 2003; Crowe and Bourchier, 2006). In contrast, other studies on multiple biological controls found no interactive effect (Kok et al., 1996; Kluth et al., 2001) or a synergistic and/or mutualistic effect (Supkoff et al., 1988; Baudoin et al., 1993; Friedli and Bacher, 2001; Caesar, 2003; Seastedt et al., 2007). As such, it is often difficult to predict whether positive, negative, or no interaction will result from the release of multiple biological control agents on a target species.

Although there have been several insects and a plant pathogen released for YST control, few published studies have evaluated interactions among them (Clement and Sobhian, 1991). Yacoub (2005) observed that there was potentially a competitive relationship between *E. villosus* and *C. succinea*. Swope (2009) examined the interaction of *P. jaceae* with *E. villosus*; however no studies have evaluated the potential interaction between the YST rust and the two most common insect biological control agents in a single system. Our objective was to determine the effect of *P. jaceae* both on YST seedhead development and attack rates of *E. villosus* and *C. succinea*.

## 2. Materials and methods

The interactive effect of *P. jaceae* on *E. villosus* and *C. succinea* was examined using two experimental designs, a YST density experiment and a competition replacement series experiment. Both experiments were conducted in 2006 and repeated in 2007. In the replacement series experiment (de Wit, 1960; Harper, 1977), we evaluated the interaction of *P. jaceae* and the two biological control insects under interspecific competition between YST and wild oat (*Avena fatua* L.). In the standard replacement series, one species is grown at different proportions (ratios) with another species (Harper, 1977), but density is held constant. In this experiment, YST plants were grown at five different ratios with wild oat. Wild oat is a widespread and robust winter annual forage grass that commonly co-exists with YST in California.

### 2.1. Field site

Experiments were conducted at the University of California-Davis Plant Sciences Field Station in Yolo County. 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 at ambient indoor temperatures (20–24 °C, ~25% relative humidity) for 4–7 months until the initiation of the experiments.

The 2006 and 2007 experimental sites were located approximately 200 m apart to ensure no residual infection of *P. jaceae* spores from the previous year. The soil at the station is Yolo fine sandy loam. Prior to planting, the sites were sprinkler-irrigated to promote weed germination, followed by harrowing several weeks later. The 2006 study area was treated with Round-up Pro® (glyphosate, Monsanto Company, St. Louis, MO) to kill emerged weeds, and the 2007 area was treated with a mix of Round-up Pro® and Surflan® (oryzalin, Dow AgroSciences, Indianapolis, IN) prior to planting. In both years, the field sites were fenced to prevent mammalian herbivory.

### 2.2. Seedling growth and outplanting

YST and wild oat seeds were sown in flats in a greenhouse in December 2005 to early January 2006 and in early January 2007. Seedlings were transplanted to the field in late January or early February (2006) or early February (2007) at the 3–6 leaf stage. In the field, the plants were sprinkler-irrigated as needed. In 2006, due to dry, warm conditions in February, 50 mm of irrigation water was provided and in the drought year of 2007, approximately 90 mm was water was added between December and March. Both the plots and the buffer rows were hand-weeded on a weekly basis 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 with seedlings of the same size maintained in the lath house before inoculation, but not afterward.

### 2.3. Experimental design

Two experimental designs were used, each 1 $m^2$ (1 m × 1 m) plots, to assess the effect of *P. jaceae* on YST and the biological control insects. Both designs were separated by 1 m bareground buffers on all sides in 2006 and 2 m buffers in 2007. In the first design, monocultures of YST were established at three densities (5, 16, and 64 plants $m^{-2}$). There were two treatments consisted of plants inoculated with *P. jaceae* and non-inoculation controls, and plots were arranged in a randomized complete block design with five blocks in 2006 and four blocks in 2007.

For the competition experiment, seedlings were planted at a constant density of 36 plants $m^{-2}$ on a 20-cm grid. Yellow starthistle and wild oat were established at the following ratios (YST:wild oat), 0:100, 25:75, 50:50, 75:25, and 100:0, with eight plots at each ratio inoculated with *P. jaceae* in a randomized complete block design with four blocks.

### 2.4. Inoculation

Plots were inoculated at the beginning of March in both years, and re-inoculated at the end of March in 2006. March inoculations have been shown to be the optimal time for *P. jaceae* infection (Fisher et al., 2007, 2008).

*Puccinia jaceae* urediniospores were provided by the California Department of Food and Agriculture (CDFA) Biocontrol Program in Sacramento. Spores were stored at −70 °C and then transferred to a standard freezer for approximately a week prior to inoculation. Spores of *P. jaceae* were applied at a concentration of 500 mg spores $liter^{-1}$ in a solution containing 0.06% of the emulsifier Tween 20® (polyoxyethylene sorbitan monolaurate, EM Sciences, Gibbstown, NJ). A $CO_2$ backpack sprayer with a two nozzle boom was used to disperse the spores at a rate of 923 liters $hectare^{-1}$ (100 gal $acre^{-1}$). Control plots were sprayed with the same solution and volume, but without *P. jaceae* spores.

Previous studies found that infection levels were greatest when newly inoculated plants were subjected to 12 h of dew (Woods et al., 2004; Fisher et al., 2007). Therefore, treated plots were individually tented with black plastic on a one foot tall frame immediately following inoculation to increase the dew potential and keep the area moist, and the plastic tents removed the following morning with at least 16 h of tenting. The time from *P. jaceae* inoculation until the appearance of pustules ranged between 10 and 21 days. Thus, plots were evaluated after three weeks to determine whether re-inoculation was required. In 2006, all plots were re-inoculated, whereas only four plots, all in the replacement series, required re-inoculation in 2007.

### 2.5. Fungicide

When non-inoculated plots showed early symptoms of *P. jaceae* infection, Quadris® (azoxystrobilin, Syngenta Crop Protection, Greensboro, NC) at 70 g/ha (10 oz/ac) was applied to these plots to minimize contamination. Non-inoculated plots were treated 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 study site to test for the effect of the fungicide on YST growth. No differences occurred in any measured parameter between fungicide-treated and control plants (O'Brien, 2008). A subsequent greenhouse experiment was also established in 2007 to test for fungicide effects on YST, with results were similar to those of the field study (O'Brien, 2008).

### 2.6. Insect introductions

*Chaetorellia succinea* naturally colonized the field sites in both years, presumably invading from nearby populations. *Eustenopus villosus* was also present early in the season but in low numbers. To ensure an adequate population of both insect species, approximately 60 mature *E. villosus* insects from the surrounding area were collected and added to the field site in June of both years.

### 2.7. Seedhead processing

Above-ground biomass was harvested in all plots in July when YST was in full flower. Harvested biomass was allowed to dry under ambient conditions for one week. Total seedheads m$^{-2}$ at or beyond the spiny stage (four or more spines fully extended at 90°) were estimated by counting seedheads from 150-gram subsamples. Mature seedheads (with petals) were also recorded, while counting seedheads, to estimate the effect of *P. jaceae* on seedhead maturation. In addition, 20 mature seedheads from each subsample were randomly selected and dissected to evaluate attack rates of the two insect biological controls. When fewer than 20 flowering seedheads were present in a subsample, additional seedheads were collected at random from that plot.

Seedheads attacked by *E. villosus* contained chambers with frass, callus tissue, and/or larvae or pupae. Because female *E. villosus* weevils drilled holes in the seedheads, it was possible to record maternal preferences between *P. jaceae* inoculated and non-inoculated plants. For *C. succinea*, it was only possible to distinguish damage caused by the emerging larvae. Therefore, data recorded for *C. succinea* was an indicator of larval infestation rate and not specifically attack rate. Because *C. succinea* larval damage in dried YST seedheads is commonly used to determine its attack habit (V. Popescu, personal communication), we refer to percentage of seedheads with damage caused by each insect as 'attack rate'.

### 2.8. Data analyses

Nominal logistic regressions were used to analyze the attack rates as presence/absence for both insects combined, and for each species. These values were then converted to total seedheads attacked, as well as total *E. villosus* and *C. succinea* attacks per m$^2$. Data were analyzed using the pair-wise contrasts in an ANOVA. The proportions of seedheads attacked (for combined insects and individual insect species) were compared using an ANOVA. Main *P. jaceae* effects and interaction terms (rust $*$ density or rust $*$ ratio) were compared using pair-wise contrasts. Data were analyzed by year and across years. All data were analyzed using the statistical program JMP© (SAS Institute, 2005).

## 3. Results

*Puccinia jaceae* infection levels for the different experiments were previously reported (O'Brien et al., 2010). Though there was some infection of control plants both years of each experiment, the inoculated treatments always had significantly higher infection rates.

### 3.1. Seedhead production and maturation

In each experiment and both years of the study, the number of mature seedheads m$^{-2}$ was not significantly different between treatments (Tables 1 and 2). For the replacement series, YST inoculated with *P. jaceae* had a 35% increase in mature seedheads, compared to total seedheads compared to control plots in 2007, and there was a 20% increase when data for 2006 and 2007 were combined (Table 1). Data were combined across years, as both years showed similar variance. However, in the replacement series experiment in 2006 and the density experiment in both years, there were no significant differences in the proportion of mature

**Table 1**

Means for growth parameters and insect attack rates from yellow starthistle and wild oat replacement series experiments for 2006, 2007 and the combination of the two years. Values are given for inoculated (I) and non-inoculated (N) plots at each ratio of YST to wild oat, and then combined for the main *Puccinia jaceae* effect over all ratios. Statistical differences (for I vs. N within a given ratio) denoted as follows: $P < 0.1$ (*), $P < 0.05$ (**). Comparisons without asterisk indicate no statistical difference. All statistical analyses performed using pair-wise contrasts in ANOVA.

| Parameter | YST:wild oat ratio | | | | | | | | Combined ratio data | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 25:75 | | 50:50 | | 75:25 | | 100:0 | | | |
| | I | N | I | N | I | N | I | N | I | N |
| *2006* | | | | | | | | | | |
| Mature seedheads (SH) (m$^{-2}$) | 126 | 217 | 323 | 281 | 305 | 387 | 460 | 571 | 304 | 364 |
| Proportion mature to total SH (m$^{-2}$) | 0.13 | 0.18 | 0.18 | 0.14 | 0.18 | 0.17 | 0.19 | 0.19 | 0.17 | 0.17 |
| Total mature SH attacked (m$^{-2}$) | 93 | 154 | 226 | 168 | 210 | 197 | 226 | 331 | 189 | 213 |
| Total *E. villosus* attacks (m$^{-2}$) | 75 | 126 | 191 | 138 | 177 | 139 | 189 | 217 | 158 | 155 |
| Total *C. succinea* attacks (m$^{-2}$) | 25 | 67 | 91 | 45 | 55 | 81 | 64 | 137 | 59 | 83 |
| *2007* | | | | | | | | | | |
| Mature seedheads (SH) (m$^{-2}$) | 634 | 349 | 247 | 555 | 533 | 394 | 686 | 780 | 525 | 520 |
| Proportion mature to total SH (m$^{-2}$) | 0.25* | 0.15 | 0.28 | 0.24 | 0.37** | 0.24 | 0.35 | 0.30 | 0.31** | 0.23 |
| Total mature SH attacked (m$^{-2}$) | 595 | 345 | 244 | 485 | 466 | 369 | 635 | 702 | 485 | 475 |
| Total *E. villosus* attacks (m$^{-2}$) | 565 | 321 | 197 | 416 | 352 | 299 | 522 | 507 | 409 | 386 |
| Total *C. succinea* attacks (m$^{-2}$) | 121 | 122 | 86 | 189 | 186 | 138 | 261 | 336 | 164 | 196 |
| *2006 and 2007 combined* | | | | | | | | | | |
| Mature seedheads (SH) (m$^{-2}$) | 380 | 283 | 285 | 418 | 419 | 390 | 573 | 676 | 414 | 442 |
| Proportion mature to total SH (m$^{-2}$) | 0.19 | 0.16 | 0.23 | 0.19 | 0.27* | 0.20 | 0.27 | 0.24 | 0.24** | 0.20 |
| Total mature SH attacked (m$^{-2}$) | 344 | 249 | 235 | 327 | 338 | 283 | 430 | 517 | 337 | 344 |
| Total *E. villosus* attacks (m$^{-2}$) | 320 | 224 | 194 | 277 | 264 | 219 | 355 | 362 | 283 | 270 |
| Total *C. succinea* attacks (m$^{-2}$) | 73 | 94 | 88 | 117 | 121 | 109 | 163 | 236 | 111 | 139 |

**Table 2**
Means for growth parameters and insect attack rates from yellow starthistle (YST) density experiment for 2006, 2007, and the combination of the two years. Values are given for inoculated (I) and non-inoculated (N) plots at each density of YST, and then combined for the main *Puccinia jaceae* effect over all densities. Statistical differences (for I vs. N within a given density) denoted as follows: $P < 0.1$(*). Comparisons without asterisk indicate no statistical difference. All statistical analyses performed using pair-wise contrasts in ANOVA.

| Parameter | Plant density per 1 $m^2$ plot | | | | | | Combined density data | |
|---|---|---|---|---|---|---|---|---|
| | 5 | | 16 | | 64 | | | |
| | I | N | I | N | I | N | I | N |
| *2006* | | | | | | | | |
| Mature seedheads (SH) ($m^{-2}$) | 463 | 604 | 740 | 521 | 875 | 774 | 693 | 633 |
| Proportion mature to total SH ($m^{-2}$) | 0.25 | 0.26 | 0.21 | 0.23 | 0.17 | 0.20 | 0.21 | 0.23 |
| Total mature SH attacked ($m^{-2}$) | 236 | 356 | 340 | 271 | 324 | 395 | 300 | 341 |
| Total *E. villosus* attacks ($m^{-2}$) | 185 | 247 | 237 | 198 | 193 | 240 | 205 | 229 |
| Total *C. succinea* attacks ($m^{-2}$) | 65 | 121 | 148 | 78 | 166 | 163 | 126 | 121 |
| *2007* | | | | | | | | |
| Mature seedheads (SH) ($m^{-2}$) | 506 | 391 | 721 | 480 | 310 | 294 | 512 | 388 |
| Proportion mature to total SH ($m^{-2}$) | 0.17 | 0.18 | 0.28 | 0.24 | 0.26 | 0.25 | 0.24 | 0.22 |
| Total mature SH attacked ($m^{-2}$) | 435 | 371 | 642 | 408 | 267 | 261 | 448 | 347 |
| Total *E. villosus* attacks ($m^{-2}$) | 420 | 348 | 584 | 389 | 205 | 238 | 403 | 325 |
| Total *C. succinea* attacks ($m^{-2}$) | 147 | 74 | 180 | 96 | 93 | 59 | 140* | 76 |
| *2006 and 2007 combined* | | | | | | | | |
| Mature seedheads (SH) ($m^{-2}$) | 482 | 509 | 732 | 503 | 624 | 560 | 613 | 524 |
| Proportion mature to total SH ($m^{-2}$) | 0.22 | 0.22 | 0.24 | 0.23 | 0.21 | 0.22 | 0.22 | 0.23 |
| Total mature SH attacked ($m^{-2}$) | 325 | 363 | 474 | 332 | 298 | 335 | 366 | 343 |
| Total *E. villosus* attacks ($m^{-2}$) | 290 | 292 | 391 | 283 | 198 | 239 | 293 | 271 |
| Total *C. succinea* attacks ($m^{-2}$) | 101 | 100 | 162* | 86 | 134 | 116 | 132 | 101 |

seedheads between the inoculated and non-inoculated plots at any ratio or density (Tables 1 and 2).

### 3.2. Puccinia jaceae and insect interactions

The total number of seedheads $m^{-2}$ attacked by the two insect agents individually or in combination were not significantly different ($P < 0.05$) between treated and untreated plots in both the replacement series (Table 1) and density (Table 2) experiments in 2006, 2007, or in both years combined. Furthermore, no consistent trend occurred in the number of attacks per unit area in experiment over the two years of the study.

In the replacement series experiment, there were no differences in the percent attack rates for *E. villosus, C. succinea*, or for the two biological controls together in the combined data for each year or the combination of the two years (Table 3). While attack rates were significantly higher in inoculated plots at the 50:50 ratio (Table 3), the difference was not consistent at the other YST:wild oat ratios.

Results in the density experiment were similar to the replacement series experiment (Table 4). In this experiment, there were no significant differences ($P < 0.05$) between inoculated and non-inoculated treatments at any density for either insect. When attack rates for both insects were combined over both years, there was a significant, though minor, decrease (9%) in the attack rate in inoculated plots (Table 4).

In each experiment and in both years, most of the seedhead damage was caused by *E. villosus* (Tables 1–4). *Eustenopus villosus* accounted for 66–73% of seedhead attacks per $m^2$ and 68–75% of total seedhead attacks in the study.

## 4. Discussion

In this study, *P. jaceae* infection did not affect the total number of mature seedheads produced per unit area, but there was correlation, albeit slight, for an increase in the seedhead maturation rate in the replacement series study. Increased rate of seedhead matu-

**Table 3**
Mean percent of yellow starthistle (YST) seedheads attacked in inoculated (I) and non-inoculated (N) plots for the combination of both insects, and for *Eustenopus villosus* and *Chaetorellia succinea* individually in the replacement series experiment for 2006, 2007 and the two years combined. Values are given for inoculated (I) and non-inoculated (N) plots at each ratio of YST to wild oat, and then combined for the main *Puccinia jaceae* effect over all ratios. Statistical differences (for I vs. N within a given ratio) denoted as follows: $P < 0.1$(*), $P < 0.05$(**), $P < 0.01$(***). Comparisons without asterisk indicate no statistical difference. All statistical analyses performed using pair-wise contrasts in ANOVA.

| | YST:wild oat ratio | | | | | | | | Combined ratio data | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 25:75 | | 50:50 | | 75:25 | | 100:0 | | | |
| | I | N | I | N | I | N | I | N | I | N |
| % Seedheads attacked | | | | | | | | | | |
| *Both insects combined* | | | | | | | | | | |
| 2006 and 2007 combined | 83 | 85 | 84 | 74 | 78 | 72 | 71 | 74 | 81 | 78 |
| 2006 | 73 | 71 | 70 | 61 | 69 | 50 | 49 | 58 | 65 | 60 |
| 2007 | 94 | 99 | 99*** | 88 | 88 | 94* | 93 | 90 | 93 | 94 |
| *Eustenopus villosus* | | | | | | | | | | |
| 2006 and 2007 combined | 73 | 75 | 69 | 63 | 62 | 56 | 59 | 51 | 68 | 63 |
| 2006 | 58 | 58 | 59 | 51 | 58 | 36 | 41 | 38 | 54 | 46 |
| 2007 | 89 | 92 | 80 | 75 | 66 | 76 | 76 | 65 | 79 | 78 |
| *Chaetorellia succinea* | | | | | | | | | | |
| 2006 and 2007 combined | 19 | 33** | 31 | 25 | 26 | 28 | 26 | 33 | 26 | 30 |
| 2006 | 19 | 31 | 28 | 15 | 18 | 21 | 14 | 24 | 19 | 23 |
| 2007 | 19 | 35 | 35 | 34 | 35 | 35 | 38 | 43 | 31 | 37 |

**Table 4**
Mean percent of yellow starthistle (YST) seedheads attacked in inoculated (I) and non-inoculated (N) plots for the combination of both insects, and for *Eustenopus villosus* and *Chaetorellia succinea* individually in the density experiment for 2006, 2007 and the two years combined. Values are given for inoculated (I) and non-inoculated (N) plots at each YST density, and then combined for the main *Puccinia jaceae* effect over all densities. Statistical differences (for I vs. N within a given density) denoted as follows: $P < 0.1$ (*), $P < 0.05$ (**). Comparisons without asterisk indicate no statistical difference. All statistical analyses performed using pair-wise contrasts in ANOVA.

| | YST density | | | | | | Combined density data | |
|---|---|---|---|---|---|---|---|---|
| | 5 Plants | | 16 Plants | | 64 Plants | | | |
| | I | N | I | N | I | N | I | N |
| % Seedheads attacked | | | | | | | | |
| *Both insects combined* | | | | | | | | |
| 2006 and 2007 combined | 66 | 75 | 65 | 67 | 59 | 68 | 64 | $70^{**}$ |
| 2006 | 51 | 59 | 46 | 52 | 37 | 51 | 45 | 54 |
| 2007 | 86 | 95 | 89 | 85 | 86 | 89 | 87 | 90 |
| *Eustenopus villosus* | | | | | | | | |
| 2006 and 2007 combined | 59 | 62 | 54 | 57 | 42 | $53^{*}$ | 56 | 58 |
| 2006 | 40 | 41 | 32 | 38 | 22 | 31 | 31 | 37 |
| 2007 | 83 | 89 | 81 | 81 | 66 | $81^{*}$ | 77 | 87 |
| *Chaetorellia succinea* | | | | | | | | |
| 2006 and 2007 combined | 21 | 20 | 22 | 17 | 24 | 21 | 22 | 19 |
| 2006 | 14 | 20 | 19 | 15 | 19 | 21 | 17 | 19 |
| 2007 | 30 | 19 | 25 | 20 | 30 | 20 | 28 | 20 |

ration may reflect the reduced photosynthetic activity caused by a reduction in leaf chlorophyll levels (O'Brien et al., 2010). Plants under stress have often been reported to mature earlier compared to healthy plants (Duniway and Durbin, 1971; Paul and Ayres, 1984). Maude and Humpherson-Jones (1980) found rusts to cause 'premature pod ripening' in Brassicaceae species, and Yang et al. (1991) reported early maturation in soybean plants with increased infection of the airborne fungal pathogen *Phakopsora pachyrhizi* Syd. & P. Syd. Although the 20% increase in seedhead maturation in the replacement series experiment may be of biological interest, we postulate that it will not have a significant effect on YST populations, as reproductive output of YST is generally far in excess of that needed to reach maximum density.

In analysis of other data from these experimental plots, we showed that *P. jaceae* had no effect on any YST growth parameter in the density experiment but caused a modest reduction in YST performance in the replacement series experiment (O'Brien et al., 2010). In the competition study, infected plants had 18% less biomass, fewer leaves, and slightly reduced rosette diameters compared to uninfected plants over both years of the experiment. In a similar study, Swope (2009) showed that *P. jaceae* caused a modest reduction in YST plant height, biomass, and number of buds and inflorescences. However, this study was conducted in a coastal ecosystem of California which is exposed to higher fog, lower temperatures and increased moisture compared to the more arid Sacramento Valley. Based on our results, we concluded that these effects would have minor biological significance and are unlikely to cause major declines in YST populations, particularly in the more arid inland areas of the state (O'Brien et al., 2010).

*Eustenopus villosus* and *C. succinea* only attack young buds or developing YST seedheads (Wilson et al., 2003). In contrast, *P. jaceae* is an obligate parasite that primarily infects the leaves of the plant (Woods and Villegas, 2004). Therefore, there is little to no direct contact between the insect agents and *P. jaceae*. Furthermore, there were no dramatic effects on the combined attack rates of the two insect biological control agents and *P. jaceae* did not interfere with the activity of either insect on YST. Indirect interactions between biological controls can occur when pathogens cause leaf chlorosis, reduce plant nutrient levels, or chemically alter the host plant (Barbosa, 1991; Stout et al., 2006). These changes in the host plant could increase or decrease insect development or attack rates. For example, Friedli and Bacher (2001) found that the larvae of stem borer weevils (*Apion onopordi* Kirby) were larger on Canada thistle (*Cirsium arvense* (L.) Scop.) host plants infected with *Puccinia punctiformis* (Str.) Rohl. Similarly, Caesar (2003) reported better control of leafy spurge (*Euphorbia esula* L.) when root-boring flea beetles (*Aphthona* spp.) were present with plant pathogens. Alternatively, Australian weevils (*Oxyops vitiosa* Pascoe) preferred to oviposit on non-rust infected *Melaleuca quinquenervia* Cav. (Rayamajhi et al., 2006).

In a study similar to ours, Swope (2009) evaluated the interaction between *P. jaceae* and *E. villosus*. She showed that the insect biological control had no preference for either infected or uninfected plants. However, because infection with the rust reduced the total number of YST seedheads, weevil attack rates were proportionally higher on *P. jaceae*-infected plants compared to non-inoculated control plants. She concluded that, in combination, *P. jaceae* and *E. villosus* have a greater than expected negative effect on YST. In contrast to her study, we did not show a dramatic negative effect of *P. jaceae* on YST growth (O'Brien et al., 2010) nor did we find any evidence for interaction between *P. jaceae* and the attack rates of *E. villosus* or *C. succinea*. The difference in the response of YST to *P. jaceae* in the two studies may be due to the location of the research sites. While our study site was located in the drier and hotter Central Valley of California, she evaluated the effect of the rust in the cooler and moister Central Coast of California. It is possible that coastal regions are more conducive to the pathological activity of the rust, which may account for the increased effect on YST.

From our study, the proportion of YST seedheads attacked in 2006 and 2007 were not statistically different between *P. jaceae*-infected and uninfected plants. Based on these results, we conclude that in the warmer and drier regions of California, *P. jaceae* does not significantly influence preference or activity of either *E. villosus* or *C. succinea* on YST.

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