## Effects of Seeding Rates and Rice Water Weevil (Coleoptera: Curculionidae) Density on Damage in Two Medium Grain Varieties of Rice

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## Abstract

Rice water weevil (*Lissorhoptrus oryzophilus* Kuschel) is a common pest of rice production in the United States whose larvae cause yield loss by feeding on the roots. We conducted studies from 2011–2013 on M-202 and M-206, two commonly grown California medium grain rice varieties, to determine if M-206 demonstrated tolerance to rice water weevil damage. Observations from field studies suggested the possibility of a level of tolerance in M-206 that was more prevalent at high seeding rates. We did this study using two different experimental units, open and ring plots. In both units, we quantified grain yields across four levels, 56, 112, 168, and 224 kg/ha, of seeding rates to detect potential yield recovery by M-206. In the open plots, we used naturally occurring weevil populations compared with controls that reduced the populations with insecticides. In the ring plots, we tested three levels of weevil infestation, none, low, and high, to look at the weevil density effects on yield and scarred plants. Our studies showed that M-206 and M-202 had generally similar densities of immature weevils and yield. Compensation for yield loss did not occur at higher seeding rates. These results suggest that M-206 does not have the ability to tolerate rice water weevil damage better than M-202. There was weak evidence that the number of scarred plants increased as plant density was reduced. The results are discussed in relation to the utility of this study to grower choices of varieties for long-term rice water weevil management.

Key words: Lissorhoptrus oryzophilus, Oryza sativa, host plant resistance, yield recovery, tolerance

Rice water weevil (*Lissorhoptrus oryzophilus* Kuschel) is the most destructive invertebrate pest of rice (*Oryza sativa* L.) production in the United States. The weevil causes yield losses up to 25% in untreated situations (Reay-Jones et al. 2008). The damage comes from larvae feeding on roots in the submerged soils (Zhang et al. 2004). The adults inflict minor injury by consuming leaf tissues, creating diagnostic longitudinal scars along the leaf blade (Stout et al. 2002). In California, scars were formerly used to gauge the intensity of an infestation with mixed results (Espino 2012). However, the presence of scarred plants may still be useful for indicating if an insecticide treatment is needed (Morgan et al. 1989, Grigarick 1992).

Common methods for mitigating damage from this pest include insecticides and delayed planting, but these methods come with drawbacks. Insecticides are effective against rice water weevil, but have nontarget effects on the aquatic invertebrates that form the basis of aquatic food webs and that can contribute to mosquito control (Lawler and Dritz 2005, Barbee and Stout 2010). Delayed planting can be effective in Texas, Louisiana, and Arkansas (Espino et al. 2009, Thompson et al. 1994), because of the use of drill-seeded systems. California rice growers rarely use drill-seeding and delayed planting because of weed pressures. California growers use continuous flooding and water-seeded systems to cope with weeds (Aghaee and Godfrey 2014)

An alternative strategy is to use host plant resistance to reduce yield loss from weevil injury. Host plant resistance relies on physiochemical characteristics of the plant to deter, tolerate, or neutralize pests to reduce damage. Since the early 1960s, researchers have screened thousands of rice lines for resistance to weevil damage, but with very little success across the United States (Bowling 1963, Gifford and Trahan 1975, Smith and Robinson 1982, N'guessan et al. 1994a,b). In the early 1980s, researchers in California identified a genotype that was tolerant to rice water weevil damage with acceptable agronomic traits (Grigarick and Way 1982, Grigarick et al. 1986); however, breeding eventually ended in 2000 following few gains in agronomic traits, such as yield (Godfrey 2001). Currently, in California, the rice water weevil is managed using insecticides. There are few cultural practices that can reduce infestations or damage, such as levee weed control, laser leveling of fields to reduce the area associated with levees where weevils overwinter, and winter flooding (Flint et al. 2013). However, two aspects of rice production may offer possibilities to manage rice water weevil infestations, variety selection, and seeding rate.

A significant change in rice production during the past decade has been the displacement of the long-time standard medium grain variety M-202 by M-206, an improved medium grain Calrose type variety now grown in over 50% of the acreage in California (Saichuk 2014). Unfortunately, most of the research regarding yield effects of the rice water weevil in California was conducted using M-202 or even older varieties. Interestingly, recent studies in commercial rice fields have failed to find significant yield reductions in M-206 due to rice water weevil infestation (Espino 2012). Additionally, small plot studies found that M-206 supported larval densities two to three times higher than M-202; at the same time, yield loss in M-206 was 330 kg/ha, while yield loss in M-202 was 1,300 kg/ha (Godfrey 2013).

Typically, small plot research on rice water weevil in California has been conducted using a seeding rate of 112 kg/ha, but commercial seeding rates average 180 kg/ha. Growers tend to use higher seeding rates (up to 224 kg/ha) when they perceive a high risk of stand and tiller reduction due to seedling pests such as tadpole shrimp (*Triops longicaudatus* LeConte) or rice seed midge (*Cricotopus sylvestris* (F.) and *Paratanytarsus* spp.), environmental constraints, or other factors. A high number of established seedlings are also useful to mitigate weed establishment. Additionally, the cost of rice seed is not a large component of overall production costs ranging from US\$37/hectare in 2012 to US\$34.5/hectare in 2015 (Greer et al. 2012, Espino et al. 2015). Over this period, the recommended seeding rates have ranged from 201 kg/ha in 2012 to 185 kg/ha in 2015 (Espino et al. 2015).

These changes in varietal preferences and seeding rates raise the question of whether they have an effect on mitigating rice water weevil damage. Rice varieties differ in biochemical and physiological properties that may affect the severity of infestation and injury by the pest (Stout et al. 2009). For example, rice water weevil adults detect plants by light reflectance and slight differences in leaf orientation could influence susceptibility (Lupi et al. 2013). High seeding rates may compensate for the yield loss from weevil damage by reducing the dependence on tiller production to achieve a high yield (Stout et al. 2009). In Louisiana, Stout et al. (2009) found damage by rice water weevil increased with lower seeding rates.

This study was designed to determine if seeding rates for two rice varieties played a role in rice plant response to rice water weevil damage. The primary objective was to determine if M-206 was tolerant to rice water weevil damage compared with M-202. Evidence for tolerance would be determined if we observed the following: M-206 with higher yields that supported populations of immatures that were higher or similar to M-202, i.e., decreased yield loss per weevil larvae. Our second objective was to determine if rice water weevils had a preference for low-density stands. Finally, the third objective was to determine if increasing the seeding rate for M-202 and M-206 would reduce yield loss from damage caused by rice water weevil larvae.

#### Materials and Methods

#### **Experimental Setup**

The study was conducted at the Rice Experiment Station near Biggs, CA, on the same tract of land from 2011–2013. The hypotheses

were tested using both open field plots and smaller ring plots designed to answer different parts of our questions. Open plots measured  $18 \text{ m}^2$  and were infested naturally by rice water weevil adults. Variables evaluated in the open plots were the effects of rice variety (M-202 or M-206), and seeding rate (56, 112, 168, or 224 kg/ha), and two levels of insecticide treatment (treated or untreated). Their infestation patterns would provide information on rice water weevil preferences for rice variety and plant density as well as plant response to weevil infestation.

However, naturally occurring rice water weevil infestation levels are variable in California, and hence, it was necessary to have experimental units where weevil populations can be controlled. Ring plots were used for this; ring plots measured 1 m<sup>2</sup> and were built using roofing metal flashing (45 cm high) with the bottom edge embedded into the flooded soil. Ring plots tested the effects of variety (M-202 and M-206), seeding rate (56, 112, 168, and 224 kg/ha), and adult weevil population levels (high, low, and none). Three ring plots were placed on the north end of each open plot that were not treated with an insecticide. This reduced the area of these plots to  $9 \text{ m}^2$ . All plots were in a randomized complete block design and replicated six times for a total of 96 open plots and 144 ring plots (Fig. 1). All plots were water seeded on 25 May 2011, 27 May 2012, and 29 May 2013. Ring plots were infested with rice water weevil in two stages on 5 and 12 June 2011, 6 and 13 June 2012, and 10 and 17 June 2013. Water management, weed control, fertility, and other cultural practices were typical of California rice (Hill et al. 2006).

## Scarred Plants, Plant Density, and Weevil Sampling

Scarred plants were counted 19 to 21 d after seeding by counting the number of plants out of 50 randomly selected with leaf scars on either of the two newest leaves within each open and ring plot. Plant density was evaluated by counting the number of rice plants in a haphazardly selected  $0.1\text{-m}^2$  area, delineated by a  $0.1\text{-m}^2$  plastic ring, of each open and ring plot at ~2 wk after planting. In 2011, the number of tillers per similar size area at the time of harvest was used to represent plant density, but in 2012 and 2013 the number of plants emerging out of the water was used to accurately reflect the density of the rice stand.

Rice water weevil immatures (larvae and pupae) were sampled annually 5 and 7 wk after seeding (WAS) from both open and ring plots by taking five cylindrical soil cores measuring 10 cm diameter by 12 cm depth. Each soil core sample contained at least one rice plant and the surrounding rhizosphere. Samples were placed in plastic bags and preserved in cold storage  $(-20^{\circ}C)$  for later processing. Samples were thawed and processed with each core washed through a 2-mm sieve to catch weevil larvae and pupae (Way and Espino 2014).

#### **Grain Yields**

Open plots were harvested using a Sweco harvester (Sweco Products Sutter, CA). A strip 0.9 m wide by either 2.7 or 5.5 m long (depending on if the plot was reduced in size from the inclusion of the ring plots) was used. Grain weights were determined on site. Ring plots in their entirety were harvested by hand and threshed using an Almaco thresher (Almaco Nevada, IA). The output was processed through a grain cleaner. In both cases, grain moisture values were determined using a DICKEY-john Corporation (Minneapolis, MN) moisture meter. Grain yields were extrapolated to a kilogram per hectare basis at 14% moisture.



Fig. 1. Experimental field setup showing both open and ring plots.

## **Statistical Analyses**

Scarred plants, stand counts, immature counts, and grain yield data from open plots were analyzed using three-way factorial ANOVAs within years with the following factors: variety, seeding rate, and treatment. Ring plot data were analyzed with three-way factorial ANOVAs within years with the following factors: variety, seeding rate, and infestation level.

Scarred plants were analyzed with SAS 9.3.1 (SAS Institute 2010) using Proc Glimmix model with a Poisson distribution and a correction factor to account for overdispersion in the data. If count data were not over dispersed or random but still failed to meet assumptions of normality they were square root ( $\sqrt{x+0.5}$ ) transformed and then analyzed using Proc Mixed with block as a random factor. Immature counts were analyzed with Proc Glimmix model with a negative binomial distribution because the data were continuous after subsamples were averaged. If the models converged and had F-values of zero, then the three-way interaction was dropped to allow a better model fit. This happened with scar data from ring plots for 2011 and 2013 and immature data from open plots in 2012 and 2013. We used the Kruskal-Wallis one-way test for single factor analysis with open plot immature counts in 2012 and 2013. We were unable to analyze two-way interactions for immature counts in 2012 and 2013 open plots for each sampling period. We opted to analyze the total sampling average for both years using a logistical regression to make sure at least the two-way interactions were not significant.

Yields and stand counts were analyzed using a normal distribution with Proc GLM. Yield data that failed the Shapiro–Wilk test but had close to normal predicted versus residual plots were winsorized, which moves outliers to within 95% and 5% of the treatment means to restore assumptions of normality. This was done because most of the outliers were from plots with much lower than average yields due to damage from rats (*Rattus norvegicus* Berkenhout) or tadpole shrimp (*T. longicaudatus*). Stand count data that failed the Shapiro–Wilk test and homogeneity of variances were square root ( $\sqrt{x} + 0.5$ ) transformed to restore assumptions for normality. Treatment means were separated using Tukey's HSD test (P < 0.05). Untransformed means are presented in the Tables 1–3, and all subsequent figures. *F*-values, degrees of freedom, and *P*-values are presented in Tables 4–6. Results from the separate logistic regressions for open plot immature counts in 2012 and 2013 are presented in Table 7.

## Results

#### Plant Density and Scarred Plants

Plant densities were consistent across seeding rates in both open and ring plots, increasing with densities ranging from 6 to 50 plants per  $0.1 \text{ m}^2$ . Scarred plants ranged from 0 to 38 scarred plants per 50 plants.

#### Open Plots

A three-way interaction between seeding rate, variety, and insecticide treatment for plant density was found in 2013 (F = 3.61; df = 6, 75; P = 0.0170). Analyzing by simple effects found that both varieties at the highest seeding rate that went untreated had higher densities (33 plants per square meter) than plots planted at the lowest

Table 1. Plant density, percent scarred plants, number of imma-<br/>tures per core sample, and yield averages in open and ring plots,<br/>2011

Treatment	Plant density (tillers per 0.1 m <sup>2</sup> )	Percent scarred plants	Immatures per core sample (5 WAS)	Immatures per core sample (7 WAS)	Average immatures per core sample	Yield (kg/ha)
			Open plots			
Variety						
M-202	63.3	18.5 <sup>a</sup>	0.31	0.23	0.27	7,237
M-206	59.9	$15.5^{b}$	0.19	0.28	0.24	7,385
Seeding rat	e (kg/ha)					
56	$55.6^{b}$	21.8 <sup>a</sup>	0.15	0.39	0.27	7,073
112	63.1 <sup>ab</sup>	$16.1^{b}$	0.23	0.27	0.25	7,478
168	61.4 <sup>ab</sup>	$14.8^{b}$	0.27	0.23	0.25	7,605
224.30	66.3 <sup><i>a</i></sup>	$15.3^{b}$	0.37	0.12	0.24	7,093
Insecticide						
Untreated	64.8 <sup><i>a</i></sup>	18.8 <sup><i>a</i></sup>	$0.47^{b}$	$0.43^{b}$	0.45	7,496
Treated	58.5 <sup>b</sup>	$15.1^{b}$	0.04 <sup>a</sup>	0.07 <sup>a</sup>	0.05	7,127
			Ring plots			
Variety						
M-202	57.3	27.9	0.69	0.63	0.66	6,471 <sup>b</sup>
M-206	62.2	27.7	0.59	0.52	0.55	7,109 <sup>a</sup>
Seeding rat	e (kg/ha)					
56	52.5 <sup>c</sup>	23.7 <sup>a</sup>	0.48	0.46	0.47	6,498
112	56.1 <sup>bc</sup>	32.1 <sup>a</sup>	0.78	0.88	0.83	7,208
168	64.2 <sup>ba</sup>	23 <sup>a</sup>	0.59	0.63	0.61	6,623
224	66.1 <sup><i>a</i></sup>	32.5 <sup>a</sup>	0.72	0.36	0.54	6,832
Infestation	level					
None	60.3	$0.8^{a}$	$0.03^{b}$	$0.02^{b}$	0.03	7,171 <sup>a</sup>
Low	57.0	36.5 <sup>a</sup>	0.86 <sup>a</sup>	0.83 <sup><i>a</i></sup>	0.84	6,323c
High	62.0	46.25 <sup>a</sup>	1.04 <sup><i>a</i></sup>	$0.87^{a}$	0.96	6,877 <sup>b</sup>
	-=		2.5.			-, -, -, -, -, -, -, -, -, -, -, -, -, -

Letters within each treatment and plot type that are the same are not significantly different according to the Tukey's HSD test (P < 0.05).

\* Denotes interactivity.

seeding that were treated (14–17 plants per square meter). Plant density increased with seeding rate in 2011, 2012, and 2013 (Tables 1–3), but was lower in insecticide-treated plots in 2011 (Table 1). M-202 had higher plant densities than M-206 only in 2012 (F=6.07; df=1, 75; P=0.0161).

Across the years, the number of scarred plants declined from ~17% in 2011 to only a trace amount (~3%) in 2013. Scarred plants were affected by seeding rate and variety only in 2011. The number of scarred plants was highest at the 56 kg/ha seeding rate in the in 2011. Insecticide-treated plots had fewer scarred plants only in 2011 (F=7.22; df = 1, 75; P=0.0089).

#### **Ring Plots**

In 2012, the variety by seeding rate interaction was significant for plant density (F = 3.12; df = 3, 115; P = 0.0288). At higher seeding rates, M-202 had higher stand counts than M-206 by up to 30% (Table 2).

Just as in the open plots, scarred plants were much lower in 2012 and 2013 than in 2011. In 2011, there was a seeding rate by infestation interaction for scarred plants (F=5.86, df=6, 121, P < 0.0001). The number of scarred plants increased as infestation level increased at the 224 kg/ha seeding rate. However, at the lower seeding rates the there was no difference in the number of scarred plants between the low and high infestations (Fig. 2). No interactions were present in other years. Other trends were that plant density increased as seeding rate increased in by ~20% in 2011 and by

Table 2. Plant density, percent scarred plants, number of imma-
tures per core sample, and yield averages for open and ring plots
2012

Treatment	Plant density (stand counts	Percent scarred plants	Immatures per core sample (5 WAS)	Immatures per core sample (7 WAS)	Average immatures per core sample	Yield (kg/ha)
	per 0.1 m <sup>2</sup> )		(2	(		
			Open plots			
Variety						
M-202	26.5 <sup>a</sup>	8	$0.004^{a}$	0.013 <sup>a</sup>	0.01	9,609
M-206	$23.8^{b}$	5.7	$0.15^{b}$	$0.12^{b}$	0.13	9,243
Seeding rat	e (kg/ha)					
56	16.8 <sup>c</sup>	7.8	0.10	0.08	0.09	8,538 <sup>b</sup>
112	$24^{b}$	6.6	0.04	0.06	0.05	9,809 <sup>a</sup>
168	28.2 <sup>ba</sup>	5.3	0.08	0.08	0.08	9,583 <sup>a</sup>
224	31.8 <sup>a</sup>	7.8	0.08	0.05	0.06	9,773 <sup>a</sup>
Insecticide						
Untreated	23.5	7	0.08	0.07	0.07	8,613 <sup>b</sup>
Treated	26.9	6.7	0.08	0.06	0.07	10 <b>,</b> 238 <sup><i>a</i></sup>
			Ring plots			
Variety						
M-202	27.1 <sup>a</sup>	16.7	0.53	0.26	0.39	7,213
M-206	$22.1^{b}$	16.2	0.60	0.30	0.45	7,320
Seeding rat	e (kg/ha)					
56	15.2 <sup>c</sup>	$16.2^{b}$	0.43	0.24	0.33	7,173
112	$22.5^{b}$	14.3 <sup>b</sup>	0.52	0.34	0.43	7,558
168	28.3 <sup>a</sup>	$14.1^{b}$	0.60	0.23	0.41	7,275
224	32.4 <sup>a</sup>	21.2 <sup><i>a</i></sup>	0.72	0.31	0.51	7,060
Infestation	level					
None	24.1	$5.7^{b}$	0.02 <sup>c</sup>	$0.00^{b}$	0.01	7,474
Low	24.6	20.4 <sup>a</sup>	$0.66^{b}$	0.38 <sup>a</sup>	0.52	7,147
High	25.1	23.2 <sup>a</sup>	$1.02^{a}$	0.45 <sup>a</sup>	0.74	7,179

Letters within each treatment and plot type that are the same are not significantly different according to the Tukey's HSD test (P < 0.05).

 $\sim$ 50% in 2013. The number of scarred plants were also higher in infested rings in 2012 and 2013 (Tables 2 and 3).

#### **Immature Counts**

Rice water weevil populations ranged from 0 to 9 immatures per core sample in open plots. On average, immature populations were higher at 7 WAS than at 5 WAS across all years in open plots (0.45 and 0.39 immatures per core, respectively). In the ring plots, the opposite was true with more immatures at 5 WAS (1.35) than at 7 WAS (0.86), and the range of immatures per core was also higher from 0 to 22 immatures per core.

## **Open Plots**

In open plots, there were no interactions among seeding rate, variety, and insecticide treatment for immature densities for all years of the study. Insecticide treatment effectively decreased the number of immatures in 2011 and 2013 with an average reduction of 68% (Table 1 and 3). In 2012, rice water weevil populations were very low (<0.1 per sample) and were not affected by insecticide treatment, but more immatures were collected from M-206 than from M-202 ( $\chi^2 = 11.38$ , df = 1, *P* < 0.0007).

## **Ring Plots**

Just as in open plots, there were no interactions among seeding rate, variety, and infestation treatment for immature densities for all

**Table 3.** Plant density, percent scarred plants, number of imma-<br/>tures per core sample, and yield averages for open and ring plots,<br/>2013

Treatment	Plant density (stand counts per 0.1 m <sup>2</sup> )	Percent scarred plants	Immatures per core sample (5 WAS)	Immatures per core sample (7 WAS)	Average immatures per core sample	Yield (kg/ha)
			Open plots			
Variety			• •			
M-202	24.5	3.5	0.15	0.20	0.17	8,943 <sup>a</sup>
M-206	24.9	3.0	0.20	0.12	0.16	7,610 <sup>b</sup>
Seeding rat	e (kg/ha)					
56	18.1 <sup>c</sup>	3.8	0.15	0.08	0.12	7,753
112	$22.9^{bc}$	3.8	0.19	0.21	0.20	7,992
168	26.5 <sup>ba</sup>	3.4	0.24	0.11	0.18	8,590
224	31.4 <sup>a</sup>	2.0	0.11	0.23	0.17	8,771
Insecticide						
Untreated	25.0	3.1	0.30 <sup>a</sup>	$0.22^{a}$	0.26	7,937 <sup>b</sup>
Treated	24.4	3.4	$0.05^{b}$	$0.01^{b}$	0.07	8,615 <sup>a</sup>
			Ring plots			
Variety						
M-202	24.3	8.5 <sup>a</sup>	0.57	0.63	0.60	3,862
M-206	23.8	$5.7^{b}$	0.63	0.59	0.61	3,640
Seeding rat	e (kg/ha)					
56	$15.1^{c}$	7.5	0.51	0.54	0.53	3,299 <sup>b</sup>
112	$23.8^{b}$	7.0	0.48	0.58	0.53	3,650 <sup>ba</sup>
168	26.8 <sup>ba</sup>	6.4	0.62	0.71	0.66	4,075 <sup>a</sup>
224	30.5 <sup>a</sup>	7.4	0.77	0.61	0.69	3,980 <sup>a</sup>
Infestation	level					
None	24.3	0.21 <sup>c</sup>	$0.09^{c}$	0.05 <sup>c</sup>	0.07	4,287 <sup>b</sup>
Low	24.5	$8.1^{b}$	$0.61^{b}$	$0.69^{b}$	0.65	3,821 <sup>a</sup>
High	23.4	12.9 <sup>a</sup>	1.08 <sup>a</sup>	1.09 <sup>a</sup>	1.09	3,145 <sup>a</sup>

Letters within each treatment and plot type that are the same are not significantly different according to the Tukey's HSD test (P < 0.05)

years of the study. Rings infested with rice water weevil had more immatures than uninfested rings on both sampling dates across all years (Fig. 3). Immature densities at both levels of infestation were quite similar when averaged over all 3 year with an average of 1.9 for the high infestation level and 1.4 for the low.

## Grain Yield

Average yields obtained from open plots were higher than yields obtained from rings. Differences were small in 2011 and 2012, but large in 2013 (Tables 1–3). The reason for this large difference is unclear. In open plots, yields ranged from 7,312 kg/ha (2011) to 8,276 kg/ha (2013). In ring plots, yields ranged from 3,751 kg/ha (2013) to 7,276 kg/ha (2012).

#### **Open Plots**

In open plots, an interaction was significant in both 2011 and 2013. In 2011, there was an interaction between variety and seeding rate. M-206 yielded significantly more than M-202 only at the 224 kg/ha (Fig. 4). In 2013, there was an interaction between seeding rate and insecticide treatment (F = 5.11; df = 3, 75; P = 0.0028) Grain yields at the three highest seeding rates were higher in treated plots than untreated plots by as much as 20% (Fig. 5). Other trends were that yields were lowest at the 56 kg/ha compared with the three higher seeding rates in 2012. Insecticide-treated plots had 15.3% higher yields than untreated plots in 2012 and 7.9% more yield in 2013.

 Table 4. Statistical parameters for stand count, percent scarred plants, adults, immatures, and yield for open and ring plots, 2011

Treatment	Plant density	Scarred plants	Immatures per core sample (5 WAS)	Immatures per core sample (7 WAS)	Yield (kg/ha)
	Open	plots 201	1		
Variety <sup>a</sup>	2.38	6.38*	0.19	0.27	0.26
Seeding rate <sup>b</sup>	4.05**	5.29**	0.09	0.49	0.84
Insecticide <sup>a</sup>	7.91**	7.22**	5.66*	7.35**	1.56
Variety × Seeding rate <sup>b</sup>	0.3	2.29	0.12	0.21	3.58*
Variety × Insecticide <sup><i>a</i></sup>	0.5	2.28	0.16	0.12	0.02
Seeding rate $\times$ Insecticide <sup>b</sup>	1.5	1.69	0.11	0.24	0.28
$\begin{array}{l} \text{Variety} \times \text{Seeding} \\ \text{rate} \times \text{Insecticide}^{b} \end{array}$	0.28	2.11	0.11	0.04	0.41
	Ring	plots 201	1		
Variety <sup>c</sup>	3.85	0.1	0.96	0.02	7.12**
Seeding rate <sup>d</sup>	6.76***	0.1	1.04	0.72	1.69
Infestation <sup>e</sup>	1.41	1.06	29.63***	7.58***	4.32*
Variety × Seeding rate <sup>e</sup>	1.37	6.97	1.15	0.09	1.49
Variety × Infestation <sup><math>d</math></sup>	1.15	0.64	1.04	0.06	0.11
Seeding rate × Infestation <sup>f</sup>	0.44	5.86***	1.8	0.39	1.18
Variety × Seeding rate × Infestation <sup>f</sup>	0.91	-	0.59	0.24	0.47

\* = P < 0.05; \*\* = P < 0.01; \*\*\* = P < 0.001; <sup>a</sup> df = 1, 75; <sup>b</sup> df = 3, 75; <sup>c</sup> df = 1, 115; <sup>d</sup> df = 3, 115; <sup>e</sup> df = 2, 115; <sup>f</sup> df = 6, 115.

Note: degrees of freedom denominator for scar = 121.

 Table 5. Statistical parameters for stand count, percent scarred plants, adults, immatures, and yield for open and ring plots, 2012

Treatment	Plant density	Scarred plants	Immatures per core sample $(5 \text{ WAS}) \chi^2$	Immatures per core sample $(7 \text{ WAS}) \chi^2$	Yield (kg/ha)
	Op	en plots 2	012		
Variety <sup>a</sup>	6.07*	3.97	11.3827**	8.4737**	1.89
Seeding rate <sup>b</sup>	24.41***	0.71	1.024	0.122	5.10**
Insecticide <sup>a</sup>	3.25	0.06	-	0.3661	37.41**
Variety × Seeding rate <sup>b</sup>	0.77	1.42	-	-	0.61
Variety × Insecticide <sup><i>a</i></sup>	0.09	0.08	-	-	0.8
Seeding rate $\times$	0.27	0.32	-	-	0.77
Insecticide <sup>b</sup>					
Variety × Seeding	1.18	0.2	-	-	0.18
$rate \times Insecticide^b$					
	Ri	ng plots 2	012		
Variety <sup>c</sup>	11.41**	0.01	0.01	0.03	0.4
Seeding rate <sup>d</sup>	46.77***	5.33**	0.22	0.01	1.58
Infestation <sup>e</sup>	1.12	88.05***	10.40***	3.99*	1.51
Variety × Seeding rate <sup>e</sup>	3.12*	0.34	0.08	0.06	3.76*
$\text{Variety} \times \text{Infestation}^d$	0.58	0.53	0.01	0.03	0.1
Seeding rate $\times$	1.61	1.22	0.22	0.05	1.44
Infestation <sup>f</sup>					
Variety $\times$ Seeding	0.19	1.22	0.08	0.01	1.1
rate $\times$ Infestation <sup>f</sup>					

Note: Immature counts were analyzed using Kruskal–Wallis tests, numerator df same as parametric analyses

\* = P < 0.05; \*\* = P < 0.01; \*\*\* = P < 0.001; <sup>a</sup> df = 1, 75; <sup>b</sup> df = 3, 75; <sup>c</sup> df = 1, 115; <sup>d</sup> df = 3, 115; <sup>e</sup> df = 2, 115; <sup>f</sup> df = 6, 115.

#### **Ring Plots**

In 2012, there was an interaction between variety and seeding rate (F = 3.76, df = 3, 115, P = 0.0129). M-206 yielded much less than M-202 at 224 kg/ha seeding rate. Uninfested ring plots had the

Table	6.	Statistical	parameters	for	stand	count,	percent	scarred
plants,	, a	dults, imma	atures, and y	ield	for ope	en and r	ing plots	, 2013

•				• •	
Treatment	Plant density	Scarred plants	Immatures per core sample (5 WAS)	Immatures per core sample (7 WAS)	Yield (kg/ha)
	(	Open plots 2	013		
Variety <sup>a</sup>	0.05	1.08	2.503	1.4	18.71***
Seeding rate <sup>b</sup>	15.26***	2.3	0.9833	1.37	1.07
Insecticide <sup>a</sup>	0.16	0.27	17.4829**	5.51*	4.23*
Variety × Seeding rate <sup>b</sup>	0.72	0.59	-	0.81	1.24
Variety × Insecticide <sup>a</sup>	0.65	0.05	-	2.3	2.18
Seeding rate $\times$ Insecticide <sup>b</sup>	1	0.68	-	1.75	5.11**
$\begin{array}{c} \text{Variety} \times \text{Seeding} \\ \text{rate} \times \text{Insecticide}^b \end{array}$	3.61*	0.55	-	_	0.43
	1	Ring plots 20	013		
Variety <sup>c</sup>	0.05	7.71**	0.02	0.1	1.29
Seeding rate <sup>d</sup>	36.34***	1.09	0.16	0.03	6.10***
Infestation <sup>e</sup>	0.37	192.99***	9.12***	9.41***	23.11***
Variety × Seeding rate <sup><math>e</math></sup>	0.63	0.22	0.28	0.16	1.06
Variety × Infestation <sup>d</sup>	0.35	2.34	0.04	0.17	2.51
Seeding rate × Infestation <sup>f</sup>	0.32	1.21	0.23	0.07	0.98
$\begin{array}{l} \text{Variety} \times \text{Seeding} \\ \text{rate} \times \text{Infestation}^f \end{array}$	1.41	0.22	0.1	0.25	0.66

Note: Immature counts were analyzed using Kruskal–Wallis tests, numerator df same as parametric analyses.

\* = P < 0.05; \*\* = P < 0.01; \*\*\* = P < 0.001; <sup>a</sup> df = 1, 75; <sup>b</sup> df = 3, 75; <sup>c</sup> df = 1, 115; <sup>d</sup> df = 3, 115; <sup>e</sup> df = 2, 115; <sup>f</sup> df = 6, 115.

Note that error df for immature counts 7 WAS are 78.

 Table 7. Statistical parameters for immatures counts for open plots in 2012 and 2013

Treatment	Immatures per core sample 2012 χ	Immatures per core sample 2013 χ
Variety <sup>a</sup>	4.4848*	3.97
Seeding rate <sup>b</sup>	2.9508	0.71
Insecticide <sup>a</sup>	1.0109	0.06
Variety $\times$ Seeding rate <sup>b</sup>	0.3572	1.42
Variety $\times$ Insecticide <sup><i>a</i></sup>	0.0352	0.08
Seeding rate $\times$ Insecticide <sup>b</sup>	2.7313	0.32
Variety × Seeding rate × Insecticide <sup>b</sup>	-	-

\* = P < 0.05; \*\* = P < 0.01; \*\*\* = P < 0.001; <sup>a</sup> df = 1, 26; <sup>b</sup> df = 3, 26

higher yields in 2011 and 2013 than infested ring plots (Table 1 and 3). In 2011, M-206 had higher yields than M-202 by  $\sim$ 9%. In 2013, yields were lowest at the 56 kg/ha compared with the higher seeding rates (Table 3).

## Discussion

We designed this study to attempt to answer three questions: 1) Is M-206 tolerant to rice water weevil damage compared with



**Fig. 2.** Percent of scarred plants (% out of 50 plants sampled) by rice water weevil infestation levels across seeding rates for ring plots in 2011. Infestation levels are ranked within seeding rate. Bars with the same letters are not significantly different according to Tukey's HSD test (P < 0.05). Bars are being compared between levels of infestation within seeding rates.



**Fig. 3.** Average number of immatures per core sample at 5 wk after seeding for each level of infestation (Control, Low, High) across all years, 2011–2013. Bars with the same letter are not significantly different from each other using the Tukey's test (P<0.05). Comparisons are being made between infestation levels across years.

M-202? 2) Do rice water weevils prefer low density rice stands? and 3) Can yield loss due to weevils be reduced by increasing seeding rate? However, there were few indications of positive answers to any of these questions. We defined tolerance as less damage per weevil between M-206 and M-202. This could appear as M-206 having a higher number of immatures per core sample and maintaining the same or higher yields than M-202 at the same seeding rate, or having a higher yield at similar infestation levels. We found no evidence that this was the case. Higher larval densities were found in M-206 than in M-202 only in open plots in 2012, but overall numbers were extremely low. We could not find a trend that would indicate preference by weevils for low density stands, and increasing seeding rate did not reduce yield loss. Beyond a seeding rate of 112 kg/ha, yields from infested and uninfested plots were not significantly different from each other.



**Fig. 4.** Open plot yields (kg/ha) of rice varieties M-202 and M-206 by seeding rate for 2011. Bars with the same letters are the same according to Tukey's HST test (P<0.05) within variety.



**Fig. 5.** Open plot yields (kg/ha) of insecticide-treated and untreated plots by seeding rates for 2013. Bars with the same letters are the same according to Tukey's HSD test (P < 0.05). Bars are comparing treated and untreated plots across seeding rate.



Fig. 6. Open plot yields (kg/ha) of insecticide-treated and untreated plots for both varieties M-202 and M-206 by seeding rates (56, 112, 168, and 224 kg/ha) in 2013. Dashed lines are untreated plots.

#### Weevil Population Trends

In California, rice water weevil population levels exceeding one immature per core are generally considered high and can cause economic yield losses (Godfrey and Palrang 1994, Hesler et al. 2000, Espino 2012). Natural infestation in the open plots resulted in lowmoderate levels of immature rice water weevil populations in 2011 and 2013, but levels were very low in 2012 (Tables 1, 2, and 3). Artificial infestation of adult rice water weevil in rings resulted in more consistent populations of rice water weevil immatures. Averaging across years, varieties, seeding rates, and sampling times, number of immature weevil in rings were 0.04, 0.67, and 0.94 immatures per core for adult infestation levels of none, low, and high, respectively. These densities represent a range of weevil populations from noneconomic to economically important. In some cases, the numbers declined between sampling dates, which happens when the larvae decimate much of the root tissue resulting in larval starvation.

#### Does M-206 Tolerate Rice Water Weevil Damage?

M-206 is the most widely planted variety in California (Saichuk 2014), comprising ~50% of the rice acreage in 2012. M-202, an older variety, will likely will be removed from production in the near future. However, most of the research in rice water weevil management has been conducted with M-202 or even older rice varieties. Our objective was to determine if M-206 differed from M-202 when infested with rice water weevil with regard to scarred plants, number of weevil immatures, and yield response.

Results showed that differences in the number scarred plants between varieties in open and ring plots occurred only once, and even then the numbers were very low. The same was true for differences in immature counts with a difference of 0.1. None of the variety interactions for yield were observed in the open or ring plots, indicating that for each treatment or infestation level, both varieties had similar yield responses. There is very little evidence to support the assertion that M-206 is preferred by rice water weevils or tolerant to damage by the weevil larvae.

# Are Lower Seeding Rates More Attractive for Rice Water Weevil?

Research conducted in the southern United States has shown that thin rice stands are more attractive for rice water weevil than dense stands (Thompson and Quisenberry 1995, Stout et al. 2009, Bernhardt 2012). This study utilized four seeding rates, from 56 to 224 kg/ha, to obtain a range of stands. Seeding rate significantly affected stand counts in both open and ring plot studies in all years, achieving the objective of establishing thin to dense stands. During 2012 and 2013, stands were between 163-347 plants per square meter, for the lowest and highest seeding rates, respectively, for both open and ring plots. In 2011, tiller counts were utilized in place of stand and seedling counts, and this revealed a similar trend, with increased tillers as seeding rate increased in both open and ring plots (Table 1). The recommended stand to obtain an adequate yield is between 108 and 433 plants per square meter (Miller et al. 1991, Flint et al. 2013), showing that in most years our stands were adequate and within the recommended range.

If stand density had an effect on weevil infestation, then this would have been reflected in trends with scarred plants or weevil immature numbers. The open plots were the best opportunity to test this hypothesis, as any invading adults had equal opportunity to infest the various plots and seeding rate treatments. Scarred plants are an indication of adult weevil activity including probable oviposition in rice seedlings, but previous attempts to predict immature populations based on scarred plants have been mixed (Espino 2012). In our study, the scarred plants did not match well with immature populations in open plots or rings. In addition, the number of scarred plants decreased greatly in the later years of the study making conclusions difficult to reach. The three-way interaction for plant density was not a serious revelation other than confirming that there was statistical separation between plant densities in untreated plots plants at 224 kg/ha and treated plots planted at 56 kg/ha. Our results from the open plots provide weak evidence, the number of scarred plants increase at lower plant densities, the only time this occurred was in 2011.

The ring plots approached this question from a different angle because we manipulated the number of weevils across seeding rate treatments. We were looking for an interaction between seeding rate and infestation, which would indicate evidence for preference. The results confirmed that weevil presence causes leaf scarring as expected. The interaction between seeding rate and infestation did not reveal anything. The problem may lie with the fact that the weevil adults used for these experiments were collected from grower fields, and populations were especially low in 2012 and 2013 (Godfrey 2013). Regardless of the number of weevil adults, we found no evidence of any ovipositional preferences and the number of immatures never differed across seeding rates.

## Can Yield Losses be Mitigated by Increasing Seeding Rate?

Growers increase their seeding rate when they perceive high risk of stand establishment challenges or as "insurance" for achieving an acceptable stand. This practice is enabled by the low seed costs. Rice water weevils do not affect stand establishment, but we hypothesized that increasing plant density could reduce the impacts of weevil damage, by essentially diluting the effect of infestation. A higher stand reduces the amount of tillers produced per plant but allows an acceptable yield to be produced from the main plant, i.e., without tillering (Tocco and Godfrey 1998). Rice water weevil injury also reduces the amount of tillering, which in turn reduces grain yield.

As higher seeding rates reduce the importance of tillering as a yield determinant, this may also mitigate the importance of rice water weevil as a plant stressor. If this was the case, a significant seeding rate by insecticide (open plots) or infestation level (ring plots) interaction would have been detected. This would have manifested as a decrease in the yield difference between untreated and treated plots as seeding rate increased.

This interaction was only significant in open plots from 2013. However, we did not observe a meaningful trend. The only change in the difference of yields came at 168 kg/ha in M-202 when the yield difference was much larger compared with other seeding rates (Fig. 6). This is contrary to what would be expected if compensation for yield loss were to occur at higher seeding rates.

Based on our results, we would not recommend increasing seeding rate to compensate for injury by rice water weevil. Growers traditionally have used higher seeding rates in water-seeded continuously flooded rice systems to compensate for damage from other biotic agents (Chauhan et al. 2011), and these advantages may still apply. High seeding rates allow for greater shading of weeds, which makes it a very helpful tactic in dry and water-seeded rice systems (Phuong et al. 2005, Anwar et al. 2011, Chauhan and Abugho 2013).

Overall, our findings contribute to a better understanding of how the rice varieties M-202 and M-206 perform under different conditions of stand density and rice water weevil infestation. The general similarity between the responses of two varieties is a testament to the fact that M-206 has a high degree of M-202 in its parentage; it was developed to be a higher yielding variety than M-202 (Jodari et al. 2004). Our study showed that both varieties are equally susceptible to the yield-reducing effects of rice water weevil injury and should be managed similarly for this pest. In addition, the use of increased seeding rates leading to higher plant densities was not shown to mitigate the damage from this pest, and there was no evidence that this practice lead to higher infestations of soil-dwelling rice water weevil stages. There was weak evidence that the inverse, i.e., thin stands, resulted in higher levels of adult rice water weevil feeding on rice leaves. There may be other advantages in rice production to higher seeding rates, but negatively impacting rice water weevils will likely not occur.

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