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Winter flooding of California rice fields reduces immature populations of *Lissorhoptrus oryzophilus* (Coleoptera: Curculionidae) in the spring

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Abstract

BACKGROUND: In California, rice fields are flooded over the winter months (November to March) to facilitate degradation of post-harvest rice straw and to provide temporary habitat for migratory waterfowl. Prior research showed that winter flood rice fields had fewer rice water weevil (*Lissorhoptrus oryzophilus*), larvae and pupae during the rice production season than fields that were left unflooded in the winter. A series of experiments were conducted to provide further support for these trends under controlled conditions and to find a mechanism for this phenomenon.

RESULTS: Under winter flooded conditions there was a 50% reduction in populations of weevil immatures compared with the untreated control (no straw or winter flood). These same conditions corresponded to a 20% increase in the amount of silicon found in plant tissues in 2014 and a 39 to 90% decrease in methane production in the soil from 2013 to 2014, respectively.

CONCLUSION: Evidence from previous field research and these controlled studies supports winter flooding as an appropriate tactic for controlling *L. oryzophilus* populations in the spring. However, the mechanism that would explain why winter flooding adversely affects *L. oryzophilus* immatures remains unclear.
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Keywords: cultural control; integrated pest management; methane; silicon; weevil

1 INTRODUCTION

Rice water weevil, *Lissorhoptrus oryzophilus* Kuschel (Coleoptera: Curculionidae), is the most deleterious invertebrate pest in rice, *Oryza sativa* L. (Poaceae), agriculture in the United States. It is native to the southern and eastern United States, but has spread to all of the temperate rice production zones in the world.[1,2] The weevil mainly feeds on aquatic and semi-aquatic plants in the families Poaceae and Cyperaceae.[3,4] The semi-aquatic adults undergo diapause during the winter months on levees and vegetated banks.[5−7] The weevil causes yield losses of up to 25% in untreated situations.[8] *L. oryzophilus* is exclusively parthenogenetic in California, Asia and Europe and therefore only represented by females. In the southern United States, *L. orzyophilus* reproduces sexually, with male and female individuals.[9]

The adults inflict minor damage by consuming leaf tissues, leaving diagnostic longitudinal scars along the leaf blade.[10,11] The larvae feed on the roots and survive in the flooded rhizosphere. They are able to breathe by using the rice plant's aerenchyma cells, which conduct gas exchange between the roots and the atmosphere.[12,13] Root feeding reduces the number of root hairs and prunes the roots, which reduces tillering and yield.[14]

Current management of *L. oryzophilus* in California relies heavily on pyrethroid insecticides, which are toxic to aquatic organisms, such as fish and benthic macroinvertebrates.[15−17] Alternative strategies for management include removing weeds on levees, delayed planting, delayed flooding and controlling flood depth.[18−21] Winter flooding can complement other integrated pest management tactics if it is shown to be effective and subject to manipulation by growers.

1.1 Origins of winter flooding

Growers developed winter flooding to degrade post-harvest rice straw without burning. Historically, a majority of growers in the Sacramento Valley area would burn their fields to dispose of straw, which had the additional benefits of reducing fungal disease incidence by killing the resting structures (esclerotia) and returning potassium to the field.[22,23] However, burning straw contributed to air quality concerns, which prompted legislation that began to reduce this practice in the early 1990s.[24]

Today, many growers maintain flooded fields in winter from November to March to aid in breakdown of the straw and to provide vital habitat for waterfowl.[25−27] Fields are later drained

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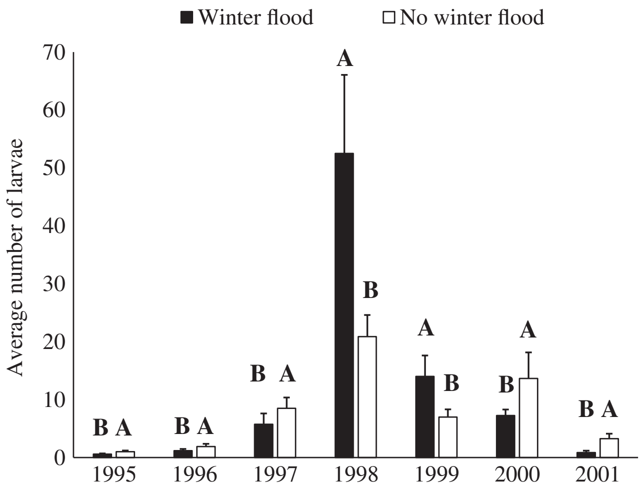

Figure 1. Average number of larvae from winter flood field experiments at Maxwell, California, for 1995–2001. Results for 1998–1999 were marred from the El Niño storm system which flooded control fields repeatedly over the course of the experiment. Overall, the data showed that winter flooded plots had lower numbers than the non-flooded fields. Bars with the same letter are not significantly different from each other within year ($P < 0.05$).

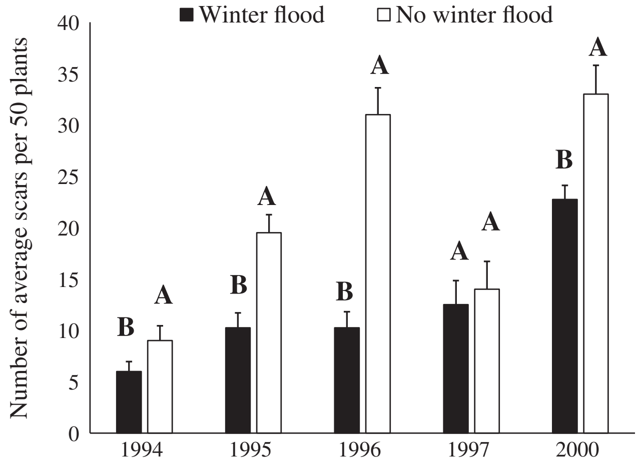

Figure 2. Average number of leaf scars per 50 plants for 1994–2001. The number of adult feeding scars is consistently smaller than in non-flooded plots. Bars with the same letter are not significantly different from each other ($P < 0.05$).

and tilled from February to March. The fields are flooded and seeded in April and May. Previous studies have examined how winter flooding affects soil characteristics, rice yield, weed management and mosquito management.[28–30] An unpublished study from 1995–2001 examined the effect of winter flooding on *L. oryzophilus* populations. That study was conducted in 0.25 acre replicated plots and reinforced with paired tests in grower fields comparing winter flooded and neighboring non-flooded fields. These studies showed that winter flooded fields had fewer weevil larvae in the following spring except in years where El Niño interfered with the treatments (Figs 1 and 2).[31] No explanation was given for the mechanisms of winter flooding affecting weevil populations. However, examination of the literature suggests that changes in paddy soil chemistry that affect microbial activity and uptake of plant nutrients, such as silicon, could possibly explain the phenomenon.

1.2 Possible mechanisms

1.2.1 Anoxia-related effects

Flooding soil depletes atmospheric oxygen and causes rapid consumption of any compound with oxygen groups, such as molecular oxygen, nitrate, sulfate and phosphate, by aerobic microbes.[32,33] The depletion in oxygen levels causes the soil microbial community to shift from an aerobic to an anaerobic community. These anaerobic microbes feed on soil organic carbon and nitrogen to produce various compounds of concern, such as carbon dioxide and methane gas.[34–36] The methane gas is consumed by methanogens or released into the atmosphere by diffusion through the rice plant.[37]

The breakdown of rice straw contributes to an increase in the amount of soil carbon.[38] This means increased methane emissions along with other anoxia-related phenomena (e.g. increased N_2O production).[39] These conditions may possibly impact *L. oryzophilus* directly through the production of methane that could interfere with weevil respiration as it diffuses through the rice plant. Alternatively, winter flooding may impair them indirectly through other anoxia-related changes in the soil microbial community. Methane production in the soil can be used as a metric for methanogenic activity by microbes.[40]

1.2.2 Silicon

Silicon is a beneficial non-essential plant nutrient. It has been shown to upregulate and prime plant defense pathways against insect pests and fungal diseases.[41–43] Rice is very dependent on the availability of silicon for growth during the reproductive stage. The majority of the silicon is translocated into the leaves in the form of silicic acid.[44,45] It is stored in phytoliths within the plant cell wall, which makes it more difficult for fungal spores to penetrate, and for manidibular insects to feed on the plant.[46] The slow release of silicon from post-harvest rice straw could help rice plants resist damage by *L. oryzophilus* immatures or cause direct harm to the weevils later in the growing season.[47,48]

1.2.3 Arsenical poisoning

Arsenic is a toxin that is taken up by rice plants accidentally through silicon and phosphorus root transporters.[49,50] In some cases, the redox changes in the soil cause arsenic to adsorb to the surface roots.[51,52] The arsenic released from post-harvest rice straw during the winter flood could then be available during the next rice cropping period. Arsenic has been shown to be toxic to insects.[53] The weevils could be ingesting arsenic which is adsorbed to the rice roots and transported into the plant.

1.2.4 Plant nutrients

Nutrients released during straw decomposition represent another factor possibly involved in reducing the number of weevil immatures. Previous research has shown that winter flooding with straw amendments increases early-season nitrogen content in the plant.[54,55] However, nitrogen fertilization studies in rice have shown, as in other crops, that increasing nutrients in the plant leads to greater pest problems when it comes to *L. oryzophilus*.[56]

1.3 Study objectives

The primary objective of this study was to investigate the impacts of winter flooding and rice straw degradation on *L. oryzophilus* populations in a small microcosm study and thereby provide support for the previous field studies on this topic. The secondary objective was to investigate possible mechanisms that could help to explain how and why winter flooding impacts spring populations of *L. oryzophilus* immatures. Based on the literature and previous field experiments, we hypothesized that: (1) the field effects observed with winter flooding will be replicated on populations of *L. oryzophilus* immatures on a smaller scale; (2) an increase in methane production in treatments amended with post-harvest straw will be correlated with weevil mortality; (3) straw amendments associated with winter flooding will increase the levels of silicon in the rice plants; (4) straw amendments associated with winter flooding will increase the concentration of arsenic in rice plants. To test these hypotheses, we gathered data on the populations of immatures, methane production output and the level of silicon and arsenic in the rice plant. We also measured levels of nitrogen, phosphorus and potassium to determine if their levels fluctuated with winter flooding or straw amendments; that was a tertiary part of the study.

2 MATERIALS AND METHODS

2.1 Experimental designs

In 2013 and 2014, microcosm experiments were conducted in a lathehouse at UC Davis in Davis, California. Plastic bins ($0.5 \times 0.3 \times 0.3$ m) were filled with 11.5 L of rice paddy soil (Esquon-Neerdobe Complex fine smectitic thermic xeric eqiaquerts, 40% clay, 2% organic matter content) taken from the Rice Experiment Station in Biggs, California. No fertilizers or herbicides were applied. The experiment was set up as a complete randomized design that was replicated 8 times and repeated with fresh material every year. There were a total of four treatments: control (no straw amendment and no winter flooding), straw amendment, winter flooding and combination (winter flood and straw).

2.2 Treatment details

The straw was added at a rate of 7.4 metric tons per hectare, based on assessments of straw production according to Lauren *et al.*[57] It was mixed into the soil by hand to a uniform distribution. In 2013, straw was purchased from a grower in Butte City, California, and in 2014, straw was purchased from a retail store in Woodland, California. The straw from the store had been chopped into ~1–3 cm lengths.

The winter flood was applied for 3 months from 18 February until 18 May. All the bins were flooded at the beginning of the trial to replicate the California hydrologic cycle of winter rain. The water used to fill the bins came from the on-site water facilities at the UC Davis Vegetable Research Center, which were sourced from a local well. Half the bins had the winter flood maintained at a depth of 10.2 cm for 3 months, while the other half were allowed to dry out over the course of the same time period. All the bins were occasionally subject to winter storms, in which case excess water was removed within 24 h of the storm event's end. At the end of the 3 month period, all bins were dried for 2–3 weeks, replicating practices used by growers. The soil in each bin was mixed, and clumps were broken to aerate the soil prior to flooding and planting for the growing season. All bins were flooded again in the second week of June and planted with a common variety of rice (M-202) at a rate of 111 kg ha^{-1} within the range recommended for seedling establishment in a California water-seeded system.[58] Adult *L. oryzophilus* were collected from rice fields in Biggs, California, and four adults (all parthenogenetic females) were caged in each bin in the last week of June for the remainder of the experiment. Plastic cylindrical cages were used (20 cm high and 36 cm diameter) with a 0.1 mm fabric mesh glued over the top. The bottom of each cage was embedded lightly into the mud to prevent adult escape.

All the adults were the same age and had survived overwintering. They were collected from the same site by hand off rice plants that had been planted 2 weeks prior. It was assumed that they laid the same number of eggs on average per bin. Weeds were a problem in the first year of the experiment; many bins had to be repeatedly hand weeded without disrupting the soil surface, i.e. only the weed top growth was removed and not the roots. In the second year, weeds were not a problem and were removed quickly in the first week of the experiment with very little disruption to the soil surface.

2.3 Immature counts

Soil corers were used to sample *L. oryzophilus* immature populations. The soil corer removed rice plants and the immediate soil surrounding the roots (10 cm deep and 6 cm diameter). Two cores were taken per bin at 4 weeks after planting. The cores were washed through a 200 μm sieve to retrieve and quantify larvae and pupae.[59]

2.4 Methane gas sampling and analysis

Methane production sampled from the soil was used as an indirect measure of microbial activity, i.e. increased concentrations of CH_4 were interpreted as greater microbial activity. Rhizon soil porewater samplers (Rhizosphere Research Products, Wageningen, The Netherlands) were installed in each bin, placed horizontally at a depth of 4 cm below the soil surface in the center of the bin. One Rhizon porewater sampler was inserted into each bin. Samples were taken 5 times during the cultivation period between June and July. Evacuated vials were used to extract 2 mL of soil porewater. After the samples were vortexed, 1 mL of the gas mixture was extracted and mixed in a separate vial with 15 mL of helium for processing in a Shimadzu® gas chromatograph with a flame ionization detector. Data from the chromatograph were converted to find the dissolved methane concentration in parts per million using an equation from Alberto and co-authors.[60]

2.5 Plant nutrient analysis

All plant nutrient composition analyses were conducted by the University of California Davis Analytical Laboratory in Davis, California. Their personnel conducted the tissue digestions and element analysis. Rice leaves were collected in July and used to examine the concentrations of nitrogen, phosphorus, potassium, arsenic and silicon. Twenty plants were sampled from each bin and pooled to represent the individual bin. Leaves were dried at 55–60 °C for 12 h. Leaves were ground up to pass through a 40 μm mesh using a Wiley® Mini-Mill. Silicon concentrations were determined using a nitric acid/hydrogen peroxide/hydrofluoric acid microwave digestion and analyzed by atomic absorption spectrometry (AAS) and inductively coupled plasma atomic emission spectrometry (ICP-AES) with a detection limit of 1 mg kg^{-1}.[61]

Arsenic levels were determined using digestion with nitric, perchloric and sulfuric acids and reduction of arsenate to arsenite and determined using a vapor generation inductively coupled plasma emission spectrometer (VG-ICP).[62] The method has a detection limit of approximately 0.05 ppm.

Nitrogen concentrations were determined based on extraction from a solution of 2% acetic acid. Nitrate was determined by flow injection analysis (FIA) using reduction to nitrite via a copperized cadmium column and diazotization with sulfanilamide followed by coupling with *N*-(1-naphthyl) ethlyenediaminie dihydrochloride. The absorbance of the product was measured at 520 nm.[63]

Phosphorus concentrations were determined spectrophotometrically by reacting with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex was reduced with ascorbic acid to form a blue complex that absorbed light at 880 nm. The absorbance was proportional to the concentration of phosphorus in the sample. Samples were analyzed using an automated flow injection analyzer.[64] Potassium concentrations were determined using an acetic acid extract with ICP-AES.[65] Concentrations and percentage levels per gram of plant material of these elements were compared between treatments. Results from these nutrient analyses were used to run a principal component analysis. The reason for quantifying changes in the concentrations of nitrogen, phosphorus and potassium was to determine whether the reduction in weevil immatures was mediated by changes in plant physiology.

2.6 Statistical analyses

Lissorhoptrus oryzophilus immature count data were square root transformed to achieve normality and analyzed as a two-way factorial ANOVA. Methane concentrations and plant tissue data that were non-normal were log transformed to restore assumptions of normality and analyzed as a two-way factorial ANOVA. All data were analyzed using SAS v.9.4.1.[66] Graphs present untransformed data with differences between bars shown by factorial ANOVA ($P < 0.05$).

2.6.1 Principal component analysis

Variables were subject to principal component analysis (PROC FACTOR METHOD = PRIN ROTATE = NONE in SAS) to detect any correlation between variables, which could confound the interpretation of the ANOVA. Factor scores created by the PCA were charted in a scatterplot with plot points labeled by treatment. Plots of factor patterns were created for factors that had eigenvalues greater than 1. Scree plots of the eigenvalues were used to determine the number of factors to retain. Transformed factor loadings of the principal component were used. Factor pattern coefficients created by the NFACTOR criterion were used to determine which variables contributed the most to each retained factor. This model immatures Factor1 Factor2 = block flood|straw was run in Proc GLM to see whether the single variable ANOVAs were justified and to detect any correlations between variables.

3 RESULTS

3.1 Immature counts

The effects of the treatments on counts of *L. oryzophilus* immatures were consistent in both years, although overall populations of immatures were higher in the control (no winter flood – no straw) in 2014 than in 2013. In both years, there were fewer immatures in the winter flooded treatments (2013: $F = 4.40$, df = 1, 21, $P = 0.0483$; 2014: $F = 5.15$, df = 1, 21, $P = 0.0340$). Within straw treatments, the combination (winter flood + straw) had no effect compared with the straw only (Figs 3 and 4). The winter flood and combination bins were not different from each other using the contrast statement in 2013 ($F = 0.10$, df = 1, 21, $P = 0.7578$) and 2014 ($F = 0.02$, df = 1, 21, $P = 0.8867$).

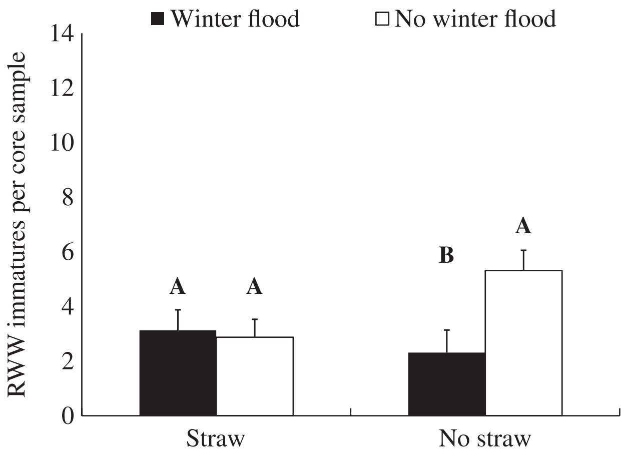

Figure 3. Average number of immatures from treatments for 2013. Bars with the same letter are not significantly different from other treatments within levels of straw ($P < 0.05$).

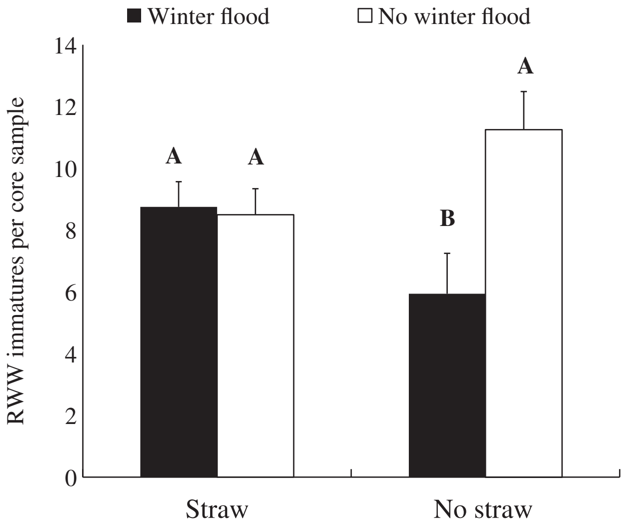

Figure 4. Average number of immatures from treatments for 2014. Bars with the same letter are not significantly different from other treatments within levels of straw ($P < 0.05$).

3.2 Methane concentrations

In 2013, winter flooding reduced methane output during the growing season ($F = 22.66$, df = 1, 21, $P < 0.0001$). The methane output in the winter flood and combination bins averaged less than 0.6 μg mL^{-1} of methane when compared with the straw and control bins which averaged 15.7 and 7.18 μg mL^{-1} respectively (Fig. 5). In 2014, the amount of methane from the bins with straw was higher. Within straw-amended bins, the winter flooded bin had produced less methane than the non-winter flooded bin ($F = 31.90$, df = 1, 21, $P < 0.0001$). No-straw treatments averaged less than 0.65 μg mL^{-1}, as opposed to straw treatments which averaged over 4 μg mL^{-1} (Fig. 6).

3.3 Plant tissue analyses

3.3.1 Silicon

In 2013 there were no differences in the silicon between treatments (Fig. 7). However, in 2014, plants in the winter flooded bin had a higher percentage of silicon per gram of dry plant material than the control bin ($F = 5.97$, df = 1, 20, $P = 0.0240$) (Fig. 8).

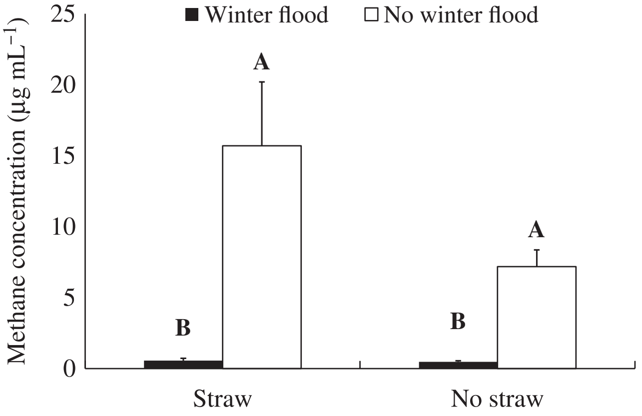

Figure 5. Seasonal methane averages (μg mL^{-1}) from treatments for 2013. Bars with the same letter are not significantly different from other treatments within levels of straw ($P < 0.05$).

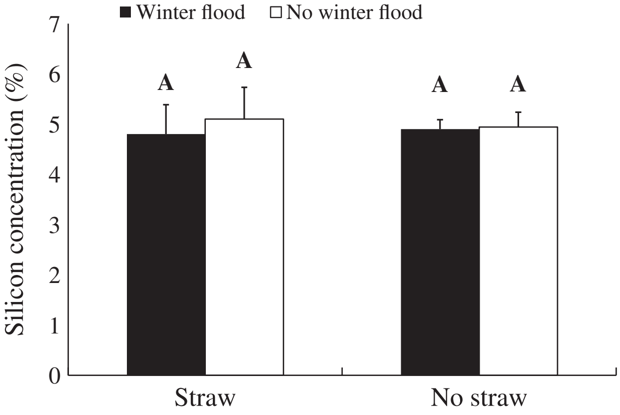

Figure 7. Silicon levels expressed as a percentage of dry plant material from treatments for 2013. Bars with the same letter are not significantly different from other treatments within levels of straw ($P < 0.05$).

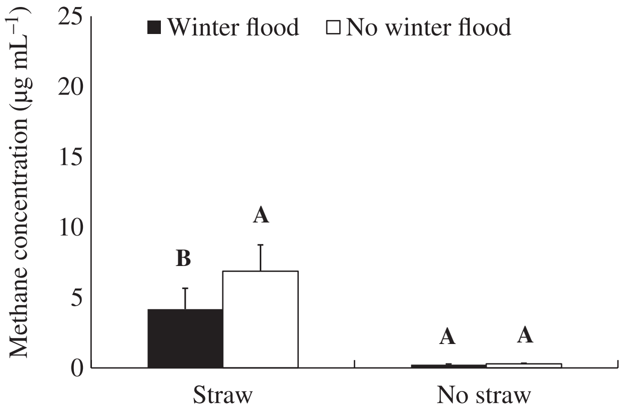

Figure 6. Seasonal methane averages (μg mL^{-1}) from treatments for 2014. Bars with the same letter are not significantly different from other treatments within levels of straw ($P < 0.05$).

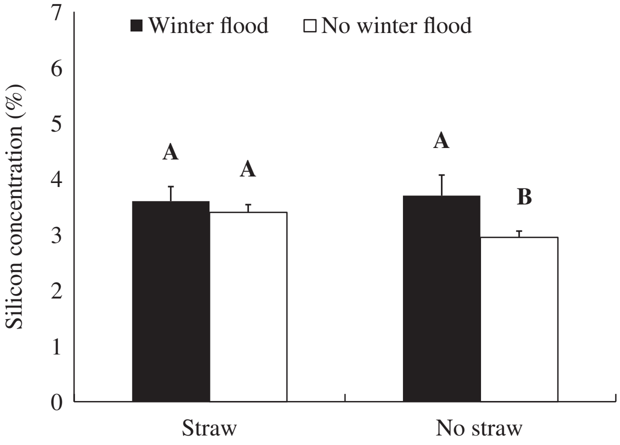

Figure 8. Silicon levels expressed as a percentage of dry plant material from treatments for 2014. Bars with the same letter are not significantly different from other treatments within levels of straw ($P < 0.05$).

3.3.2 *Arsenic*

In 2013, plant concentrations of arsenic were reduced in the winter flooded treatments ($F = 12.74$, df = 1, 20, $P = 0.0019$) by an average of 40%, regardless of the presence of straw (Fig. 9). In 2014 there were higher concentrations of arsenic (>4 ppm) in straw-amended bins ($F = 12.98$, df = 1, 20, $P = 0.0018$) compared with treatments without it (Fig. 10). There were no interactions in either year.

3.3.3 *Principal component analysis*

In 2013, two factors with eigenvalues greater than 1 were chosen, based on the scree plot of eigenvalues. The first factor was a weighted mean consisting of nitrogen, phosphorus and the strong negative presence of silicon. The second factor was a weighted mean consisting of methane concentration and arsenic. Both factors explained 70% of the variation observed in the correlation matrix. The factors were run through the model Factor1 Factor2 = block + flood + straw + flood*straw. Flood affected factor 1 (nitrogen, phosphorus, potassium and silicon) ($F = 6.91$, df = 1, $P = 0.0198$) and factor 2 (methane and arsenic ($F = 25.88$, df = 1, $P = 0.0002$). Straw had no effect on either PCA factor.

In 2014, factor 1 was composed of nitrogen, phosphorus and potassium. Factor 2 was composed of methane and arsenic. The factors were run through the previously mentioned model. The flood and straw did not affect factor 1. Straw only affected factor 2 ($F = 34.53$, df = 1, $P < 0.0001$).

4 DISCUSSION

Winter flooding in California is a practice used by growers to enhance the degradation of post-harvest straw and provide temporary wetland habitat for migratory birds of the Pacific flyway.[67] Several years of field and lathe house experiments provide support for the additional role of winter flooding of rice fields as a pest management tactic, but one that requires further improvement. Discovering the mechanism for this tactic would allow for it to be manipulated and enhanced. We found that winter flooding does reduce the number of weevil immatures, but this is only statistically significant in the absence of straw. The straw treatment by itself also reduces the number of immatures, but through a poor stand that harms the weevils. Winter flooding is also associated with reductions in methane produced in the soil during the early part of the growing season. We found that silicon accumulation was highest in winter flooded treatments without straw, and that arsenic accumulation was highest in straw treatments in 2014. We found no correlations between plant nutrient concentrations, methane production and number of *L. oryzophilus* immatures.

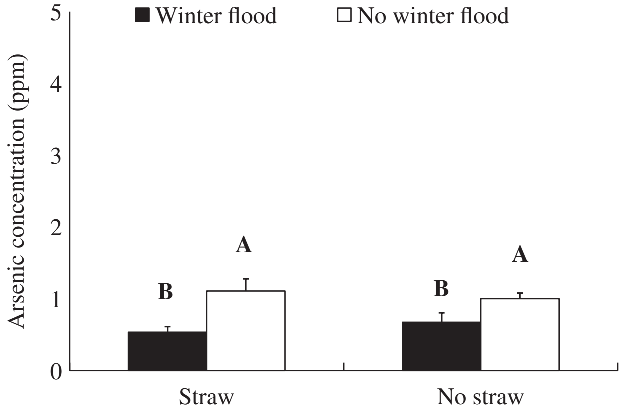

Figure 9. Arsenic levels in parts per million from treatments for 2013. Bars with the same letter are not significantly different from other treatments within levels of straw ($P < 0.05$).

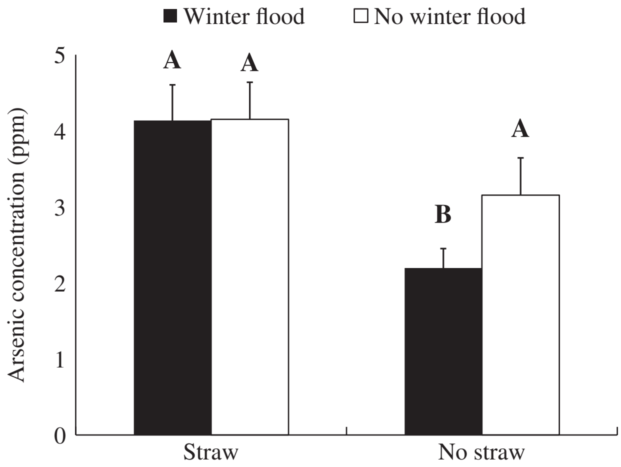

Figure 10. Arsenic levels in parts per million from treatments for 2014. Bars with the same letter are not significantly different from other treatments within levels of straw ($P < 0.05$).

4.1 Does flooding reduce weevil immatures?

Data from our 2 year experiment showed that winter flooding by itself reduced weevil immatures by 50% compared with the control (no winter flood + no straw). However, the combination was not significantly different from the winter flood treatment in 2013 or 2014. The combination treatment was different from the control in 2014. This was not expected.

In the field study from the 1990s, fields had straw incorporations and were winter flooded, and they still had a significant reduction in the number of immatures recovered in the spring compared with those that were not winter flooded. The different results between the field and microcosm studies could be explained by the fact that our microcosm study was only flooded for 3 months. In the field, growers will flood their fields for 3–6 months. The extra 1–2 months may be necessary for adequate breakdown of the straw and possibly explains the discrepancy between our microcosm experiments and the field study.

We observed fewer larvae in the straw-only treatments because of the poor stands. There was undecomposed straw at the start of the planting season, and it created a poor seedbed. Undecomposed straw at the start of the growing season increases disease incidence and reduces stands.[20] Decomposition of plant material in the soil matrix under flooded conditions releases many breakdown products and increases the level of anaerobic activity in the soil.[68] Extension recommendations for growers are to chop and incorporate straw residues into the soil or gather the straw into bales in the fall to avoid floating or drifting straw.[58] This is a problem that growers frequently face when it comes to spring residue management.

Microbial competition for nutrients with the plants could have also exacerbated the problem.[69] In a separate field study where straw was added prior to planting, all the rice plants were stunted and few larvae were found in the samples (Aghaee M-A, unpublished). However, in the second year, the rice plants in the straw-only treatments were healthier than the year before, but not as vigorous as the rice plants in bins without straw.

4.2 Methane production

In addition to our efforts to provide further support for previous field observations, we also sought to understand and explain why flooding reduced populations of weevil immatures. Given that flooding of soils promotes an anaerobic environment,[70] we expected that winter flooding would correspond to a higher level of anaerobic activity, as represented by methane production, which could be correlated with the data on the immatures. Our data showed that the opposite trend occurred; we found lower methane production in the winter flooded bins, with the exception of 2014 where both no-straw treatments had similar methane outputs. The results suggest that anaerobicity as measured by soil methane production is not a factor that affects the *L. oryzophilus* immatures in the rhizosphere. The data suggest that a different quantitative measure of microbial activity in the soil is needed.

4.3 Silicon

Silicon has been shown in the literature to improve plant defenses against mandibular insects, but not as well against phloem- and xylem-feeding insects.[71] In the second year of the study we observed more silicon in the tissues of the rice plants in the winter flooded treatments across both straw treatments. We had expected that the addition of straw during winter flood would further increase the amount of silicon available to the plant, but silicon levels were similar in both straw treatments regardless of winter flooding. Silicon released from straw during the season occurs in two releases, one during breakdown of the physical tissues and the second during the growing season from the slow release of silicic acid from the plant cell remnants.[46,72] We posit that the straw + winter flood treatment showed higher silicon in 2014 because the straw had been further processed into smaller bits, allowing it to release nutrients faster than in 2013. Therefore, the difference observed is simply a confirmation that straw decomposition releases silicon into the soil, as shown in previous studies. Measuring the content of the silicon in the parent material could have provided insight into our results. Any future studies should include an analysis of the straw prior to decomposition in the flooded soils. The silicon data were not correlated with immature counts and therefore may not be an appropriate mechanism for reducing immatures.

4.4 Arsenic

It was expected that arsenic uptake in winter flooded bins with straw could explain the decrease in *L. oryzophilus* larvae, because flooding of soil increases mobility of arsenic in the soil profile[73] that is released from the straw. Arsenic levels were low in 2013, with an average of 1 ppm observed for the entire experiment, and differences are only observed across winter flooding effect. In 2014, the average for arsenic was 4.1 ppm. However, we found no association between arsenic concentrations and the number of immatures across treatments.

There are several important notes that need to be highlighted here. The first is that the straw in 2014 decomposed faster than the year before because it had been processed into smaller pieces. No straw remnants were observed in the combination treatments in 2014, but there were plenty of strands of tissue remaining in the year prior. If there was the same amount of arsenic in the straw in both years, then more of it may have been released in 2014. Evidence from the literature shows that microbial degradation of organic matter, including rice straw, increases the availability of arsenic to the plant, mediated by conditions in the paddy soil.[74]

However, it is also possible that the straw used in 2014 had a higher arsenic content than the straw used in 2013. This may explain the higher average arsenic concentration in 2014. However, as we did not test the straw for arsenic levels prior to adding it to the experiment, we cannot assume anything about the starting concentrations of arsenic in the straw.

Secondly, silicon may have played a role in affecting arsenic concentrations in winter flooded conditions. Increased silicon uptake has been shown to reduce the uptake of arsenic in some cases. Arsenic competes with transporters in the root cells of rice plants that are specific to silicic acid.[75,76] This explanation is possible with the data from 2014, although recently published work shows that silicon from fresh straw amendments failed to reduce arsenic levels in rice plants. This may be due to the arsenic already present in the tissue relative to the silicon.[77]

However, silicon cannot be a useful explanation in 2013, where silicon levels varied 0.2% across all treatments. Arsenic levels were lower in both sets of winter flooded treatments compared with the non-winter flooded treatments by 0.4–0.5 ppm.

Given that the data are consistent in its effect in no-straw treatments across both years, a difference in soil redox potential could explain the observation. Unfortunately, no redox measurements, which could have shed light on this, were taken. When straw is present, rice plants have more arsenic accumulated in their tissues, erasing any effect that occurs in the absence of the straw in winter flooded conditions.

Thirdly, we removed weeds during the winter flood period in 2014. The weeds may have been a very important confounding factor. They may have taken up or adsorbed some of the arsenic on their roots, thereby reducing their availability to the rice plants in 2013.[78,79] For example, if the straw had higher arsenic content in 2014, then we should have observed higher plant concentrations of arsenic in bins with straw. However, we observed higher plant arsenic across all treatments. Another possibility is that high density of weeds in the soil oxygenated the soil surface enough to immobilize arsenic in the rhizosphere, or the arsenic was immobilized in the iron plaque that forms on roots in the oxidized rhizosphere.[80–83]

Finally, the LC_{50} of arsenic toxicity to kill insects is much higher than in our study. When looking at arsenic sensitivity in chironomid larvae, which are functionally similar to *L. oryzophilus* larvae, the doses required for mortality are 1–3 orders of magnitude higher.[84] It is very possible that arsenic concentrations were never high enough to cause any harm to the weevil larvae within the parameters of this study, based on the chironomid LC_{50} values. However, it would be necessary to test *L. oryzophilus* larvae for their response to arsenic toxicity to be certain.

4.5 Correlations between factors

Statistical analyses using principal component analysis showed that none of the variables that we measured could be linked with the winter flood effect on the immatures. The winter flood effect was significant for the principal components (factors 1 and 2) that corresponded to the beneficial plant nutrients and the arsenic/methane group in the first year. In the second year we saw that the straw treatment was significant only for factor 2 (methane and arsenic). When we included these factors in the model with the number of immatures, there was no effect of the treatments on the number of immatures. When we included the immatures in the principal component analysis, they were not correlated with any of the individual variables. The results of the PCA confirmed the need for the individual variable ANOVAs, and nothing of serious biological importance was revealed besides the association of plant nutrients as a single group and methane to arsenic. The association between methane and arsenic most likely indicates the common influence of soil redox potential and oxygen levels in the rhizosphere.

5 CONCLUSIONS

The mechanisms that are responsible for reducing the survivorship of weevil immatures in winter flooded fields is still an open question. Conditions that promote low levels of methane production and the uptake of silicon are those that correspond to, but not correlate with, lower weevil survivorship. The reduced methane outputs in winter flooded bins should not be taken as a sign of low anaerobic activity; it may be necessary to use a different metric, such as N_2O or even H_2 production, to measure anaerobic microbial activity.[85] If none of the soil or plant physiological processes can explain the mechanism of winter flooding as a pest management tool, then another process must be involved. The extended anoxia could be an environment where enthomopathogenic microbes thrive and attack the weevils. Environmental factors, such as temperature, humidity, soil moisture and texture, are important factors in determining the success of natural entomopathogenic microbial outbreaks and controlled applications of microbial pesticides.[86–88] However, evidence for such a phenomenon remains to be seen, and at this point in time this idea is speculative but plausible.

In the meantime, it would be prudent to investigate the timing of the winter flood and the depth of the winter flood to manipulate weevil populations in paired field and microcosm experiments.

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