ANNUAL REPORT
COMPREHENSIVE RESEARCH ON RICE
January 1, 2018 – December 31, 2018

PROJECT TITLE: The Environmental Fate of Pesticides Important to Rice Culture

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LEVEL OF 2018 FUNDING: $51,238

OBJECTIVES AND EXPERIMENTS CONDUCTED BY LOCATION TO ACCOMPLISH OBJECTIVES:

Objective I. To investigate the factors governing pesticide dissipation in California rice fields. Emphasis for 2018 will be to focus on the insecticide Coragen (chlorantraniliprole) by characterization of its degradation in soil under rice field conditions.

Objective I. Microbial Degradation of Coragen in Rice Field Soils

Introduction

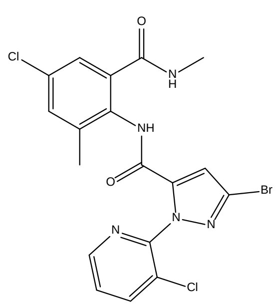

Figure 1. Chlorantraniliprole, the active ingredient of Coragen

Chlorantraniliprole (CAP, trade name Coragen, Figure 1), an anthranilic diamide insecticide, was provided supplemental labeling in 2016 for use on California rice fields through December of 2018 as a pre-flood treatment for the prevention of yield losses attributable to rice water weevil larvae. While comparatively less toxic than pyrethroid insecticides to non-target species such as crayfish and pollinators, CAP is highly toxic to aquatic invertebrate species. (USEPA, 2008; Barbee *et al.*, 2010; Dinter *et al.*, 2010; Dinter *et al.*, 2015) Furthermore, freshwater planarians and non-target soil arthropods have shown reduced activity, reproduction, and feeding after short term exposure to CAP indicating potential for ecological impacts at sub-lethal concentrations that could result from spray drift, weir leakage, or release with tailwater. (Rodrigues *et al.*, 2016; Lavtizar *et al.*, 2016) Previous investigation of the partitioning of CAP using simulated California rice field conditions concluded that CAP is unlikely to volatilize (calculated Henry's Law constant of 1.69 x 10^{-16} – 2.81 x 10^{-15} $atm \cdot m^3 \cdot mol^{-1}$ from 15 – 35 °C) and exhibits moderately weak sorption to soil ($LogK_{OC}$ = 2.59 – 2.96), resulting in an estimated 76.58 – 87.58% of the applied dose to remain in the soil. (Redman *et al.*, 2018) Therefore, the degradation of CAP in California rice field soils should be investigated to ensure adequate dissipation prior to the release of tailwater into the Sacramento River system.

Pesticide degradation in soil is dominated by the microbial community present and the properties of the local environment that influence them. When a rice field is flooded, anaerobic conditions are rapidly established with oxic regions persisting within a few millimeters of the soil surface and rhizosphere causing dramatic changes in the dominant microbial communities. (Liesack *et al.*, 2000; Gao *et al.*, 2002; Tanji *et al.*, 2003; Noll *et al.*, 2005) As a result, pesticide degradation rates under pre-flood aerobic conditions can differ greatly from those measured under flooded anaerobic conditions. (Williams *et al.*, 2017; Mulligan *et al.*, 2016; Vasquez *et al.*, 2011; Tomco *et al.*, 2010) Extended flooding periods, combined with elevated temperature and rapid evapotranspiration in California rice fields, also exacerbate the evapoconcentration of salts resulting in measured electrical conductivities exceeding 4.0 $dS \cdot m^{-1}$. (Scardaci *et al.*, 2002; Linquist *et al.*, 2015) Increased salinity has been shown to negatively impact microbial and enzyme activity in soils; however, to our knowledge, an investigation on the impact of salinity on the degradation of pesticides in soils has not been conducted. (Omar *et al.*, 1994; Rietz *et al.*, 2003; Wichern *et al.*, 2006; Sall *et al.*, 2015)

This investigation aimed to characterize the degradation of CAP in soil under simulated California rice field conditions and determine the impact of soil properties, flooding, and field salinization on degradation rates. To achieve this, degradations half-lives were determined via microcosm experiments with two rice field soils from the Sacramento Valley maintained at 50% water holding capacity (aerobic) or flooded (anaerobic) at three different salinities.

Method

The degradation of CAP in two rice field soils was studied using microcosms of 25 g of soil either maintained at 50 % WHC in loosely capped sterilized 250 mL amber polypropylene bottles (aerobic treatments) or with 50 mL of sterile water in tightly capped sterilized 250 mL amber polypropylene bottles (anaerobic treatments). Additional saline anaerobic treatments were prepared using 50 mL of 0.01 M or 0.05 M aqueous solutions of NaCl, $CaCl_2$, $MgSO_4$ and Na_2SO_4 (10:1:2:1 mol ratio). Microbe activity was inhibited in control microcosms through triplicate autoclaving. Microcosms were incubated at room temperature for 3 weeks to allow pH and redox conditions to equilibrate before spiking with 50 µL of 250 $\mu g \cdot mL^{-1}$ CAP in methanol; negative checks were prepared identically and spiked with 50 µL of methanol. Hydrolysis controls were prepared by spiking 10 mL of sterilized water in amber borosilicate vials with 40 µL of 250 $\mu g \cdot mL^{-1}$ CAP in methanol. For all treatments, microcosms and sterilized controls were sampled in triplicate along with a negative check at 0, 3, 7, 14, 21, 28, 63, 91 and 119 d after spiking. Triplicate hydrolysis controls were diluted 1:10 (v/v) with methanol at each time point prior to analysis by liquid chromatography tandem mass spectrometry (LC-MS/MS).

Results

Preliminary analysis of the results shows that CAP degradation followed first order kinetics (R^2 = 0.50 – 0.89) for the first 63 days of incubation during all treatments, after which further degradation was not observed (Figure 2). Rate constants (k_{deg}) and half-lives ($t_{1/2}$) calculated from the first 63 days of incubation for all treatments are presented in table 1. The half life of CAP ranged from 59 – 100d and 78 – 172d in native and autoclaved microcosms, respectively. Degradation was observed in the sterilized hydrolysis controls ($t_{1/2}$ = 198d); however, degradation proceeded faster in autoclaved soil microcosms, indicating that hydrolysis alone does not account for the loss observed in autoclaved treatments and that soil microorganisms are likely responsible for a significant portion of CAP degradation in these experiments.

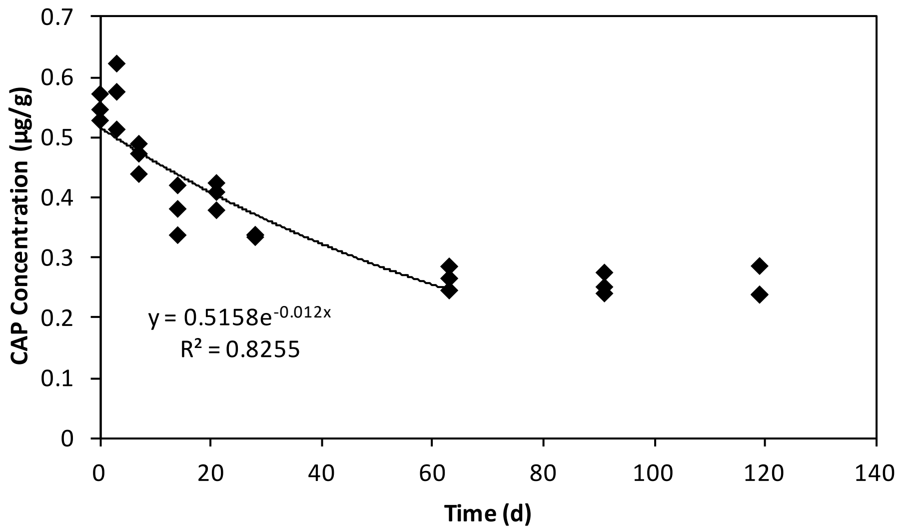

Figure 2. Representative degradation curve depicting concentration of CAP over time in a California rice field soil under anaerobic conditions with no addition of salt. Points represent individual microcosms.

Preliminary evaluation of the data by three-way analysis of variance (ANOVA), performed with JMP Pro 13 statistics software, showed no statistically significant difference between CAP degradation rates in each treatment ($\alpha = 0.05$). However, Additional statistical analysis testing that the data meet the prerequisite assumptions of the ANOVA model to ensure validity of the test as well as post hoc pair wise comparisons of treatments are required to say with complete confidence whether or not degradation is significantly different between treatments. Though, preliminarily, CAP degradation half-lives in soil are not expected to vary greatly from the observed 59-100 d based on field soil properties, flooding practices, or salinity. Soil micronutrients, such as nitrogen and phosphorus, have been shown to play an important role in the degradation of pollutants in soil. (Kanissery *et al.*, 2011) In a study evaluating the effects of ammonium based fertilization on microbial processes in a rice paddy soil, researchers observed a depletion of ammonium from non-ammended soils after 70 days. (Bodelier *et al.)* It's possible that depletion of micronutrients in the microcosms, resulting in the death or dormancy of soil microorganisms, could be responsible for the cessation of degradation observed after day 63. Future research should be conducted to determine the impact of specific soil micronutrients on the biotic degradation of CAP as well as determine the contribution of photolysis to the abiotic degradation rate of CAP in soils.

Table 1. CAP soil degradation rate constants (k_{deg}), half-lives ($t_{1/2}$) and R^2 (n = 3)

		Aerobic		**Flooded**		**Flooded (0.01M salinity)**		**Flooded (0.05M salinity)**	
Soil	**Parameter**	**Native**	**Autoclaved**	**Native**	**Autoclaved**	**Native**	**Autoclaved**	**Native**	**Autoclaved**
BS	**k_{deg} (d^{-1})**	$1.011x10^{-2}$	$8.176x10^{-3}$	$1.174x10^{-2}$	$8.833x10^{-3}$	$6.915x10^{-3}$	$4.037x10^{-3}$	$9.058x10^{-3}$	$5.174x10^{-3}$
	(±SE)	(±$8.30x10^{-4}$)	(±$9.01x10^{-4}$)	(±$1.238x10^{-3}$)	(±$7.81x10^{-4}$)	(±$9.79x10^{-4}$)	(±$9.22x10^{-4}$)	(±$8.64x10^{-4}$)	(±$8.89x10^{-4}$)
	$t_{1/2}$ (d)	68.6	84.8	59.0	78.5	100.2	171.7	76.5	134
	(±SE)	(±5.6)	(±9.3)	(±6.2)	(±6.9)	(±14.2)	(±39.2)	(±7.3)	(±23.0)
	R^2	0.89	0.81	0.83	0.87	0.72	0.50	0.85	0.64
PS	**k_{deg} (d^{-1})**	$7.495x10^{-3}$	$7.312x10^{-3}$	$6.954x10^{-3}$	$6.471x10^{-3}$	$7.544x10^{-3}$	$5.414x10^{-3}$	$8.503x10^{-3}$	$6.093x10^{-3}$
	(±SE)	(±$9.18x10^{-4}$)	(±$8.70x10^{-4}$)	(±$1.392x10^{-3}$)	(±$9.30x10^{-4}$)	(±$1.462x10^{-3}$)	(±$6.56x10^{-4}$)	(±$1.149x10^{-3}$)	(±$9.11x10^{-4}$)
	$t_{1/2}$ (d)	92.5	94.8	99.7	107.1	91.9	128.0	81.5	113.8
	(±SE)	(±11.3)	(±11.3)	(±20.0)	(±15.4)	(±17.8)	(±15.5)	(±11.0)	(±17.0)
	R^2	0.78	0.79	0.57	0.73	0.60	0.78	0.74	0.70

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SUMMARY OF 2018 RESEARCH (major accomplishments) BY OBJECTIVE:

Objective I. The degradation of chlorantraniliprole (CAP) in soil was evaluated via microcosm experiments using two rice field soils under aerobic and anaerobic conditions at varying salinities. The half-life of CAP ranged from 59 – 100 days though was not statistically correlated with soil, redox state, or salinity; however further statistical analysis testing the validity of the ANOVA model followed by post hoc comparison of the treatments is required. Further research should be conducted to determine the impact soil micronutrients have on the biotic degradation of CAP and to characterize the role of photolysis on the abiotic degradation of CAP in California rice field soils.

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CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

1. The overall goal of our ongoing research program is to characterize the dissipation of pesticides under California rice field conditions. There are generally four contributing processes are investigated: volatilization to air, sorption (bonding) to soils, and degradation by either sunlight or soil microbes.

2. For Coragen, microcosm experiments have shown that microorganisms are an important contributor to the degradation of Coragen in soils. However, observed degradation of Coragen in soil was moderately slow and preliminary analysis suggests that field soil properties, flooding practices, and salinity did not significantly influence degradation. The validity of the ANOVA model used is under evaluation and will be followed by post hoc comparisons of experimental treatments.