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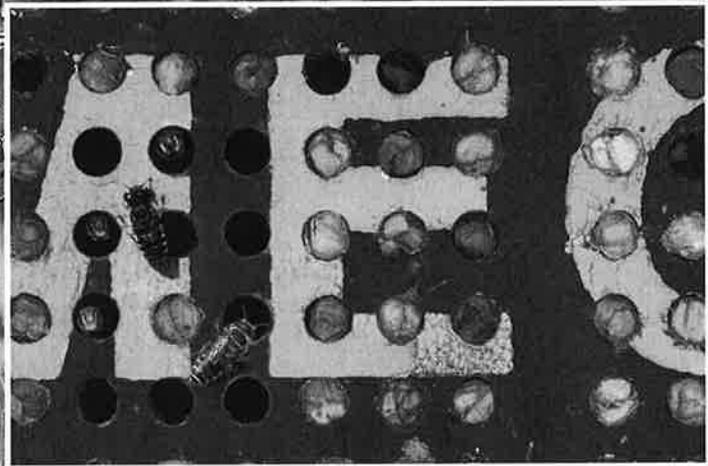
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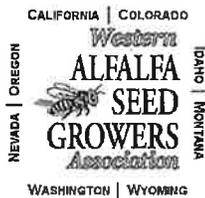
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**WESTERN ALFALFA SEED GROWERS
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Sunday, January 27, 2019

6:00 pm – 7:30 pm - Get Acquainted Reception – *Sponsored by – International Pollination Systems*

Monday, January 28, 2019

7:00 – Registration Desk Opens

Session 1

8:00 – **Conference Kickoff** – Shane Johnson, Western Alfalfa Seed Growers Association

8:10 – **USDA-RMA Alfalfa Seed Insurance Program Update** – Terrence Katzer, Risk Management Specialist, USDA-RMA, Kansas City, MO (Via Zoom Meetings)

8:40 – **Forage Starts Here: Risk Management Education for Alfalfa Seed Producers** – Keith Kennedy, Agrimind

9:40 – **Break – Raffle Drawing** – *Coffee and Breaks Sponsored by – Northstar Seed, Leafcutters*

Session 2

10:00 – **NAFA Update** – Beth Nelson, President, National Alfalfa and Forage Alliance

10:30 – **The Bayer/Monsanto Merger – Impacts on Ag** – Doug Rushing, Monsanto

11:00 – **Alfalfa Seed Production Survey (US Growers Only)** – Moderated by Jerry Neufeld, University of Idaho

12:00 – **Lunch & Raffle Drawing** – *Sponsored by – Alforex Seeds*

Session 3 – Research Session – Moderated by Teresa Pitts-Singer

1:40 – **Impact of Immune Stress on Pathogen Susceptibility & Life History Traits in *Nomia melanderi*** – Kate Hunter, Utah State University

2:00 – **Enhancing & Protecting Populations of Alfalfa Seed Pollinators** – Doug Walsh, Washington State University

2:20 – **Impact of Organosilicone Spray Adjuvants Upon Health & Reproduction of the Alfalfa Leaf Cutting Bee** – Diana Cox-Foster, USDA-ARS, Logan, UT

2:40 – **Chalkbrood Control Potential of Hypochlorous Acid in Managed Alfalfa Leafcutting Bee Populations** – Ellen Klinger, USDA-ARS, Logan, UT

3:00 – **Study of Life Cycle of *Melittobia* and *Pteromalus* in Populations of *Megachile rotundata* in Summer, Fall & Winter** – Alan Anderson, USDA-ARS, Logan, UT

3:20 – **Raffle Drawing/Announcements**

3:30 – **Presenter's Meet and Greet Social & Researchers Poster Panel** – *Sponsored by JWM Leafcutters*

5:00 – **WASGA Board of Directors Meeting**

Tuesday, January 29, 2019

Session 4

8:30 – **Welcome** – Shane Johnson, Western Alfalfa Seed Growers Association

8:40 – **Leafcutter Bee Incubation and Bee Health Study** - George Yocum, USDA/ARS, Fargo ND

9:10 – **Linking Pollinator Behavior to Gene Flow to Improve Coexistence** – Johanne Brunet, USDA-ARS VCRU, Madison, WI

9:40 – **Lygus Efficacy Trials in Alfalfa Produced for Seed** - Doug Walsh, Washington State University

10:10 – **Break – Raffle Drawing** – *Coffee and Breaks Sponsored by – Northstar Seed, Leafcutters*

Session 5 – Roundtable Discussion

10:30 – **Status of Western Hay and Forage Markets** – Josh Callen, The Hoyt Report

11:00 – **State of the Alfalfa Seed Industry** – Shawn Barnett, General Manager, Forage Genetics International

11:40 – Q&A

12:00 – **Raffle Drawing/Announcements**

12:30 – Conference Adjournment

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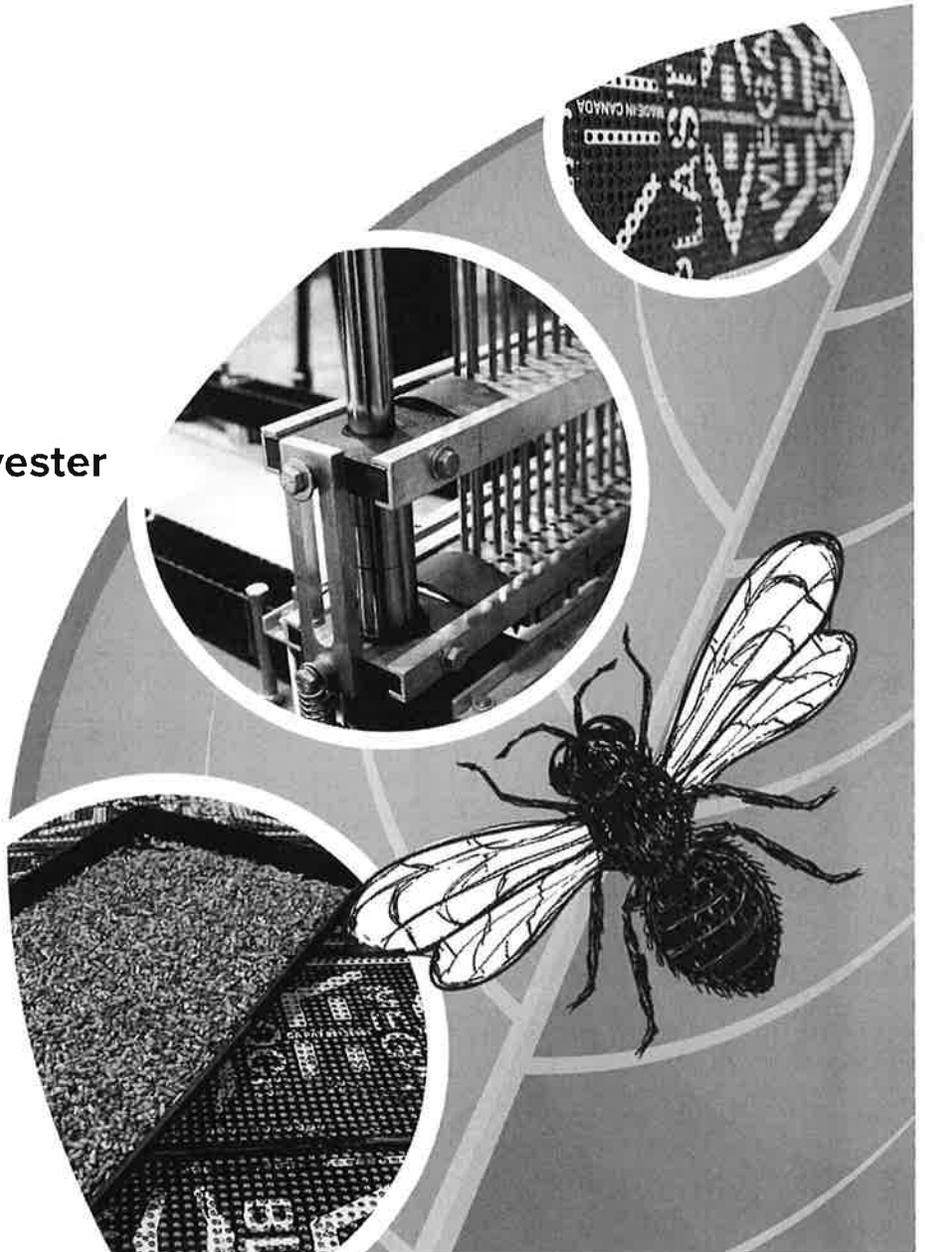
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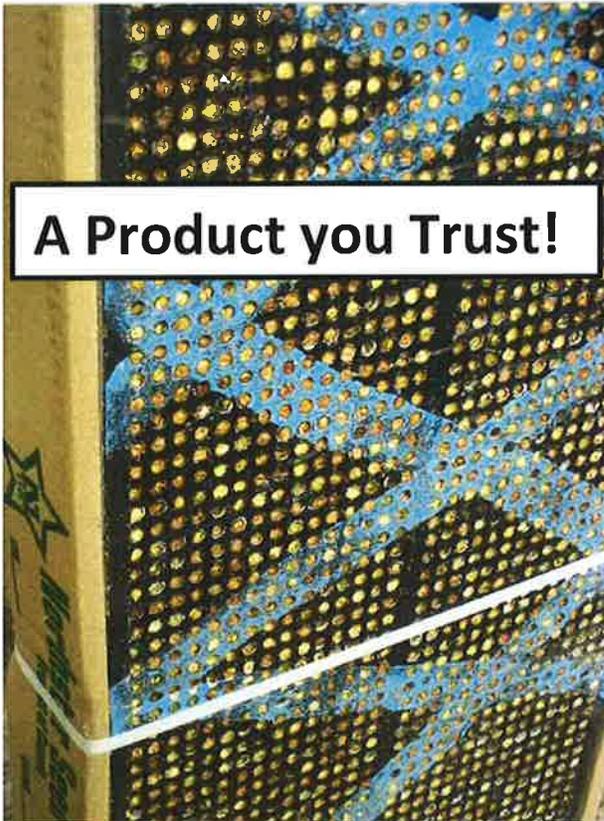
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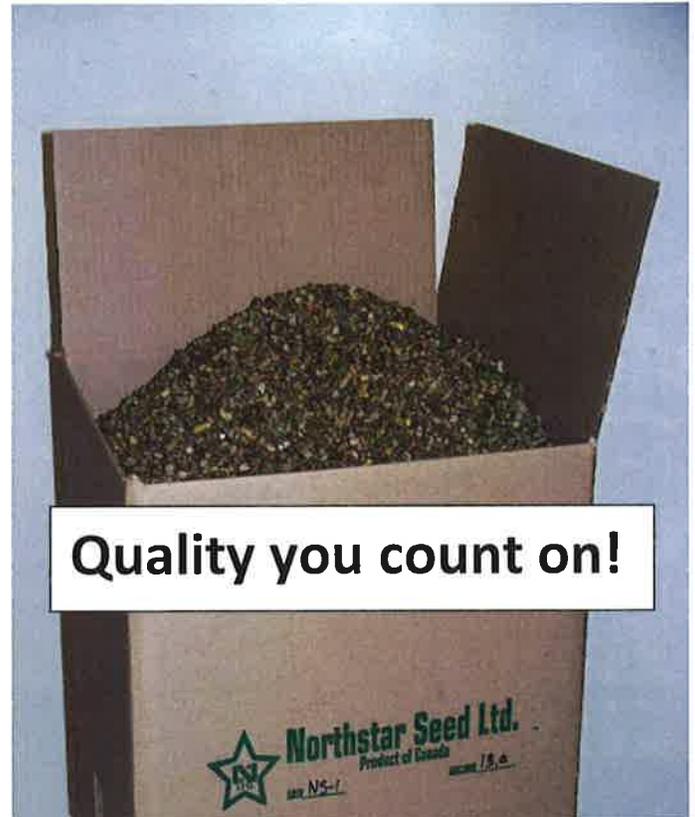


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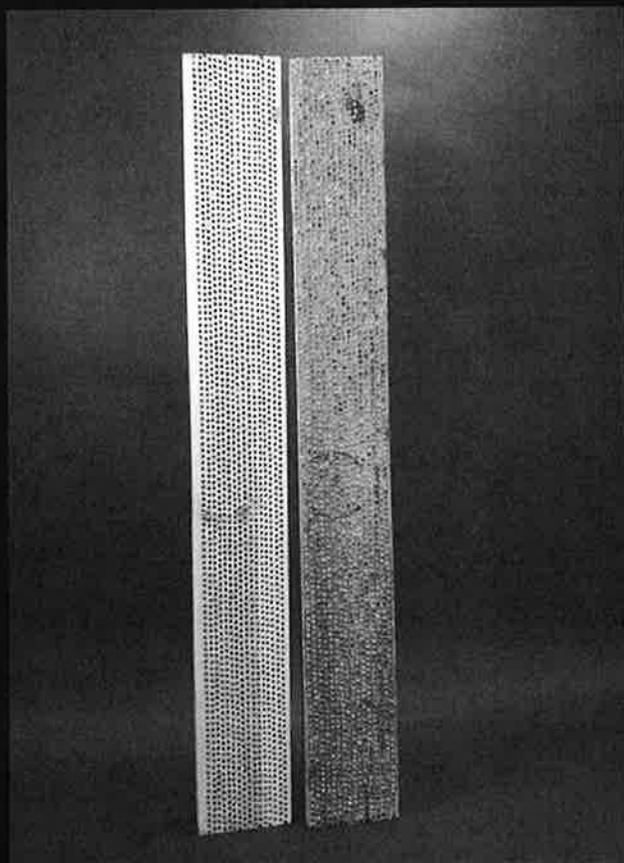
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Project Report-January 2019

The impact of immune stress on pathogen susceptibility and life history traits in *Nomia melanderi*

Principal Investigator

Karen M. Kapheim, Department of Biology, Utah State University, Logan, UT

Justification

Reproductive output and longevity are two of the most important factors in sustaining alkali bee beds and determining pollination rate. The number of offspring produced each season directly determines the number of females available for pollination the following season. Moreover, the longer each female lives and the more offspring each female produces, the more flowers they must visit to collect provisions. The first step toward implementing management strategies aimed at maximizing reproductive output and longevity in alkali bee populations is to investigate the factors that influence variation in these life history traits.

In most organisms, life history traits such as survivorship and reproduction are negatively correlated, because energetic resources invested in growth and maintenance deplete resources that can be invested in reproductive effort, and vice versa (Stearns 1992). The total energetic resources available for alkali bees to invest in reproductive effort and longevity may be reduced in the face of environmental stress, such as exposure to pathogens, climatic shifts, nutritional limitation, and exposure to pesticides, all of which potentially activate the immune system (Schwenke et al. 2016). This tradeoff has been studied in other bees. For example, bumble bees delay reproduction (Meeus et al. 2014) or have reduced lifespan (Fürst et al. 2014) when infected with different viruses. Optimizing pollinator management thus requires understanding which stressors alkali bees are regularly exposed to, the effects they have on life history tradeoffs, and the mechanisms underlying these effects.

The goal of this project is to determine the impact of immune stress on the aspects of alkali bee life history most critical to pollination services – survivorship, reproductive potential, and health (i.e., viral infections). Year 1 of the project focused on the effects of immune stress on survivorship and reproductive effort. Year 2, if funded, will focus on alkali bee health, including the effects of immune stress on viral loads and nutritional stores. This report describes results obtained from field and laboratory experiments performed in Touchet, WA in summer 2018. Results should be treated as preliminary until they are submitted for publication. All resulting publications will be sent to WASGA.

Research Objectives

- 1. Determine how alkali bees adjust their investment in reproductive effort and survivorship in response to immune challenge**

Introduction

Wild pollinators, including alkali bees, harbor titers for viruses likely to require increased energetic investment (Galbraith et al. 2018). Viral infections have been shown to impact honey bee traits related to pollination performance such as survivorship (Dainat et al. 2012) and learning (Iqbal and Mueller 2007). However, the physiology of honey bees is different from wild bees, and our ability to extend these findings to alkali bees is therefore limited. For example,

honey bee studies are usually performed on workers, which are inherently non-reproductive. It is therefore unclear how immune stress impacts investment into reproduction. Further, many wild bees live longer than honey bee workers, and it is therefore important to understand the effects of immune stress over the course of an entire lifespan. We aimed to understand the sub-lethal effects of immune stress on native pollinators, using a lab-controlled study with newly emerged alkali bees.

Methods

We used a classic experimental method to trigger an immune response without pathogenic effects in newly emerged female alkali bees. This method entails injecting heat-killed bacteria (*E. coli*) into the abdomen of anesthetized bees. We used a dose previously shown to have sub-lethal effects in honey bees, as we wanted to observe the effects of investment in immune function on longevity and reproduction without death resulting directly from the treatment (Yang and Cox-Foster 2007). Injections of heat-killed *E. coli* were paired with two controls – an injection control with only the solvent (insect Ringer’s solution) and a cold handling control (anesthetization, but no injection). To observe the effect over time, we sacrificed individuals at 1, 5, 10, and 20 days after treatment (Table 1). At the end of the treatment period, bees were frozen in liquid nitrogen and then brought back to USU for abdominal dissections. Effects on longevity were estimated by checking for dead bees three times per day throughout the treatment period. Effects on reproductive effort were determined through abdominal dissections to measure oocyte and Dufour’s gland maturation after bees were sacrificed at the end of the treatment period.

Results

We did not observe a significant effect of immune stress on longevity among newly emerged female alkali bees. Mortality was relatively low across all treatment groups (Table 1), and there were no significant differences in survival (Wald statistic=0.56, df=2, p=0.77; Fig. 1).

Table 1 Number of individuals in each treatment. Bees that died during the treatment period are indicated in parentheses. Percentages indicate survivorship.

Injection Treatments	Timespan Treatments (Day)			
	1	5	10	20
Sham	5(0)=6 100%	5(2)=7 71%	12(2)=14 86%	15(5)=20 75%
Ringer’s	7(2)=9 78%	3(3)=6 50%	13(3)=16 81%	25(5)=30 83%
<i>E. coli</i>	4(0)=4 100%	6(2)=8 75%	12(0)=12 100%	17(8)=25 68%

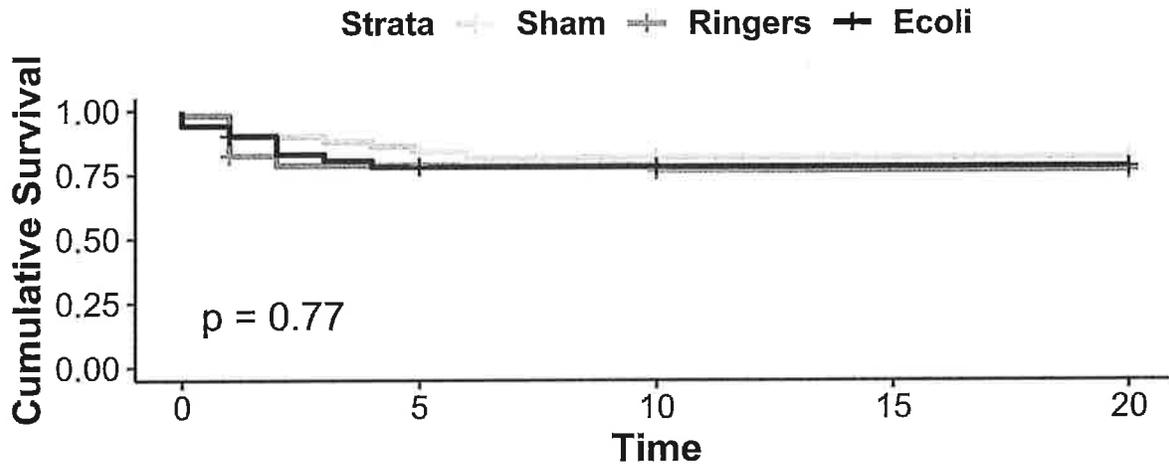


Figure 1. Survival curve showing the probability of individuals to survive in each treatment over time. There were no significant differences in survival among immune stress treatments (Wald statistic=0.56, df=2, p=0.77).

Consistent with previous research (Kapheim and Johnson 2017), female alkali bees became reproductively mature as they aged from emergence. Maximum oocyte and Dufour's gland length increased with age (oocyte length – linear model: $F=41.65$, $df=3$, $p<2.0*10^{-16}$, Fig. 2; Dufour's gland – linear mixed model: $\chi^2=126.13$, $df=3$, $p<2.2*10^{-16}$, Fig. 3). Oocyte maturation stage also increased with age (cumulative link mixed model, 5 days: $N=14$, $z=1.97$, $p=0.05$, 10: $N=37$, $z=4.23$, $p=2.37*10^{-5}$, 20: $N=57$, $z=4.49$, $p=7.25^{-6}$, Fig. 4).

Immune stress does not appear to impact reproductive maturation among female alkali bees. Neither maximum oocyte nor Dufour's gland length was significantly different among bees in different immune stress treatment groups (main effect of immune stress: oocyte – $F=0.06$, $df=2$, $p=0.94$, Fig. 2; Dufour's gland – $\chi^2=1.13$, $df=2$, $p=0.57$, Fig. 3). Immune stress treatment also did not have significant effects on oocyte maturation stage (Ringers: $z=0.55$, $p=0.58$, *E. coli*: -0.22 , $p=0.83$, Fig 4). There was not a significant interaction between immune stress and treatment period (oocyte length – $F=0.64$, $df=6$, $p=0.70$; Dufour's gland – $\chi^2=9.54$, $df=6$, $p=0.15$).

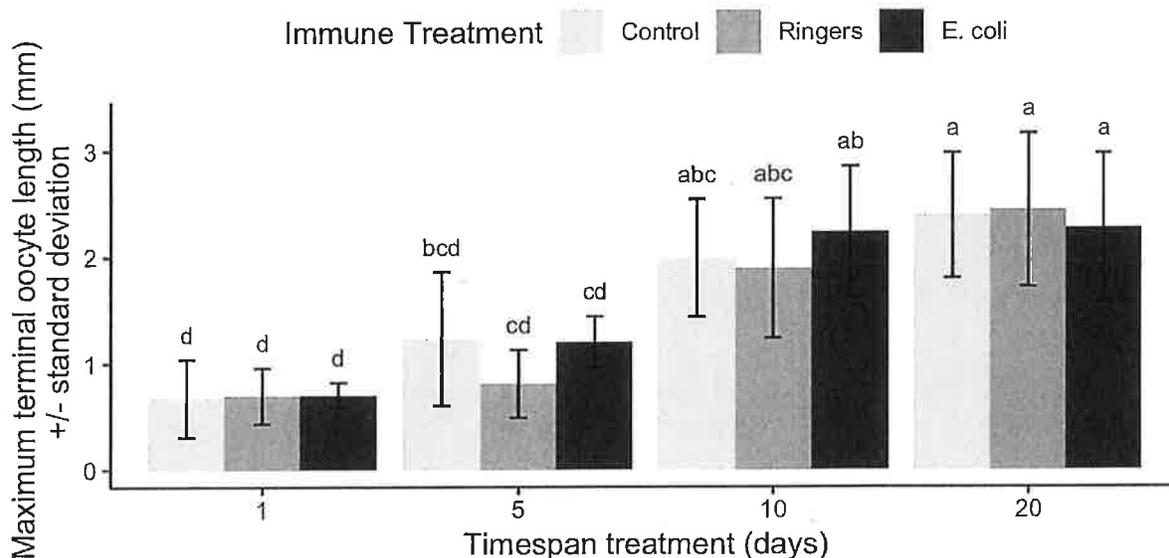


Figure 2. The impact of immune stress on oocyte length across time. Treatment with *E. coli* did not significantly influence oocyte length at any treatment length. Compact letter display shows significant differences between treatments, where groups sharing the same letters are not significantly different (generated from a Tukey HSD test).

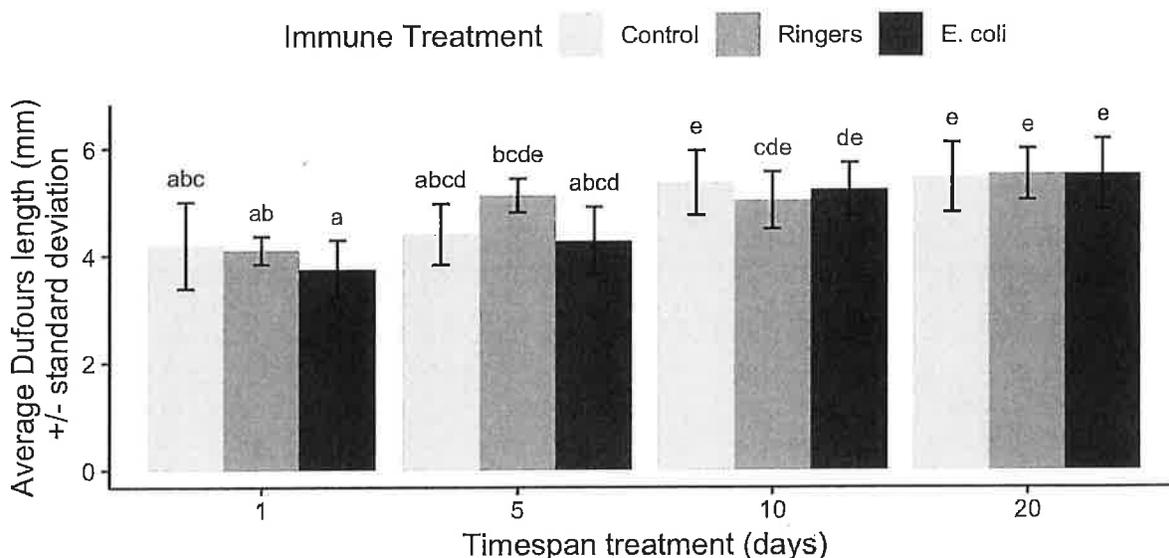


Figure 3. Impact of immune stress and timespan on Dufour's gland length. Treatment with heat-killed *E. coli* did not affect Dufour's gland length, but length increased with age (i.e., treatment length). There was also no interaction between immune stress and treatment length. Compact letter display generated using a pairwise comparison between the estimation of marginal means (or least-square means). These show significant differences between groups such that groups with a same letter are not significantly different from each other.

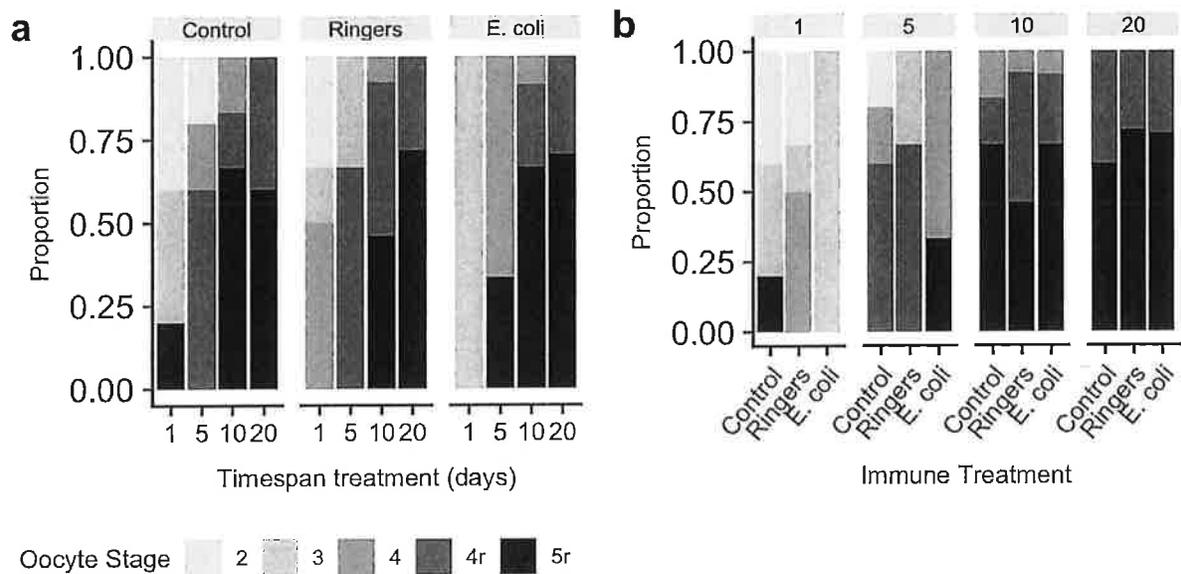


Figure 4. The impact of an immune stressor on oocyte maturation stage. A) Mosaic plot showing changes in oocyte stage over time faceted by immune treatment. B) Mosaic plot showing changes in oocyte stages in each immune treatment faceted by treatment period. Legend at the bottom represents oocyte stage, from immature to mature (2-5r). The r symbolizes the most developed oocyte being in a resorbing stage.

Discussion

Preliminary results suggest immune stress does not inhibit reproductive maturation or longevity in female alkali bees. The strongest effects of immune stress are likely to occur immediately after the treatment, when most females are still in an early stage of reproductive development (Fig. 4). It does not appear that energy invested into immunity during this stage significantly inhibits future reproductive effort. However, this is also the treatment group for which we have the lowest sample sizes (Table 1), which reduces our power to detect significant differences. Figure 4 shows that females in the control groups tend to have more mature oocytes than females treated with heat killed *E. coli* one day after the treatment, though this difference was not statistically significant. If there is an undetected significant effect of immune stress immediately after treatment, it appears females can overcome this setback within five days, when no such trend is observable (Fig. 4).

It is also possible that a higher dose of heat killed *E. coli*, and thus stronger immune challenge, might have affected longevity or reproductive maturation. However, the current dose was chosen based on previous work with honey bees (Yang and Cox-Foster 2007), and sub-lethal effects are most ecologically relevant to wild pollinator biology. These results suggest that immune stress among young alkali bees is not likely to impact reproductive effort or longevity. This could indicate that immune stress in alkali bees is not likely to place major constraints on

pollination services, but additional testing is necessary to determine whether immune stress impacts other aspects of health in wild bees.

2. Identify mechanisms underlying tradeoffs between reproductive effort, survivorship, body condition, and susceptibility to viral infection in alkali bees

We will obtain a more complete picture of how immune stress impacts alkali bee health in year 2 of the project. We will use quantitative PCR to evaluate the effects of immune stress on genes involved in nutrition and immunity, as well as to quantify viral loads. These effects have been previously shown in honey bees (Yang and Cox-Foster 2007), but are unknown for alkali bees.

3. Evaluate the frequency of viral infections and mechanisms of vertical transmission of viruses in alkali bees

In year 2 of the project, we will also characterize the frequency and transmission patterns of viruses in alkali bees. Previous research has demonstrated that alkali bees carry some of the same viruses as honey bees in Touchet, WA (Galbraith et al. 2018), but the frequency and transmission patterns of these viruses are not known.

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Project Title: Enhancing and Protecting Populations of Alfalfa Seed Pollinators

Years of Study: 2018-2019

Lead Investigator/Affiliation:

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Objectives:

1. Conduct topical direct contact bioassays with candidate pesticides on ALCB and alkali bees.
2. Test sulfoxaflor and a candidate bioinsecticide on ALCB on a large-scale (20+ acre) demonstration plot to evaluate pest control and pollinator safety at the macro scale.
3. Develop and validate an alkali bee emergence model using cost effective technology and quantifying the change in bee emergence rate achieved by irrigating soil at field margins.

Results:

Objective 1. *Conduct topical direct contact bioassays with candidate pesticides on ALCB and AB.*

In 2018 we focused on a biological insecticide called Biological Soil and Foliar and the insecticides chlorantraniprole and cyantraniliprole. The candidate insecticides were applied with a R&D CO₂ sprayer at 26 gal/A using a hand boom to 0.01-acre plots of alfalfa being produced for seed in the Lowden alfalfa seed-growing district on July 10, 2018. Field-weathered residual test exposures on each acaricide was replicated 5 times per candidate insecticide at 1 hour after the insecticides were applied. Samples consisting of approximately cutting 400 cm³ of foliage will be taken from the upper 15 cm of the plants and clipping this alfalfa to 2.5 cm lengths. This hay was then placed into individual plastic Petri dish (15 cm diameter) replicates, the tops and bottoms of which are separated by a wire screen (6.7 meshes/cm) insert (45 cm long and 5 cm wide).

Extant ALCB and AB were collected by sweep net from alfalfa fields grown for seed from screen mesh barriers placed next to AB beds or at the entrance of ALCB domiciles. The bees were tranquilized with CO₂ and put in the Petri dish bioassay cage. Bees in cages were held at 75°F for 8 hrs and mortality counts were assessed at the conclusion of this time period. Bees were considered as “living” if they were capable of flying away after the 8 hour exposure in the bioassay arena. The bees were considered as “dead” if they failed to fly away. Mortality was corrected against control bioassay arenas. Typically control mortality is about 10%.

Our results were fairly conclusive that the Insecticides Biological Soil and Foliar and the insecticides chlorantraniprole and cyantraniliprole are safe for both AB and ALCB at the maximum labeled rates (Table 1). Past research has demonstrated that less than 25% mortality in the contact bioassays in 1 hr residues is indicative that these pesticides will not have knock-down toxicity to foraging bees.

Table 1. Corrected mortality of ALCB and AB to 8 hrs of exposure to treated alfalfa foliage collected 1 hr after acaricide application.

Product	Rate per acre	ALCB % Corrected Mortality	Alkali bee % Cororrected Mortality
Biological Soil and Foliar	96 fl oz	7	4
Chlorantraniprole (Coragen)	7.5 fluid oz	4	2
Cyantraniliprole (Exirel)	20.5 fl oz	13	9

In 2017 with Biological Soil and Foliar we had promising results in our Lygus insecticide efficacy trials. In 2018 insecticide efficacy trials Biological Soil and Foliar provided absolutely no control or suppression of Lygus populations. We will include Biological Soil and Foliar in our 2019 trials but if the products performance is a poor as it was in 2018 we will eliminate it from the program.

Chlorantraniprol and cyantraniliprole (Coragen and Exirel) are classified by the Insecticide Resistance Action Committee as Group 28 insecticides. Group 28 insecticides like chlorantraniprole and cyantraniliprole are commonly called diamides and their mode of action is that the are ryanodine receptor modulators. These insecticides have nerve and muscle activity. Insect toxicologists believe that there is strong evidence that action at this protein complex is responsible for insecticidal effects of these two insecticides. Chlorantraniprole and cyantraniliprole are both registered on a number of horticultural and field crops. Chlorantraniprole tends to have more registrations targeting caterpillar pests. Cyantraniliprole has registrations that target a greater diversity of pest insects beyond caterpillars including weevils and aphids. Hence, cyantraniliprole could have usefulness in alfalfa seed production. In 2019 Walsh has been funded by the NAFA alfalfa check-off program to conduct bioassay and field efficacy studies for control of weevils in forage and seed alfalfa production. Chlorantraniprole and cyantraniliprole are both reduced risk insecticides and if we develop efficacy data it is likely that the registrant, FMC would support 24C Special Local Need registrations on alfalfa produced for seed.

Based on the contact efficacy data all three insecticides tested in 2018 would be safe for foraging bees. However, both chlorantraniprole and cyantraniliprole are highly systemic and will move through the phloem. There is a likliehood these insecticides will be found in nectar following

treatment. If either of these insecticides is efficacious against key pests further and more detailed studies on their toxicity to leafcutting and alkali bees would be warranted. Studies have been completed with honeybees and bumble bees. The results of these studies have shown that exposure via nectar feeding did not result in bee kill at field rate exposure levels.

Objective 2. Test sulfoxaflor and a candidate bioinsecticide on ALCB on a large-scale (20+ acre) demonstration plot to evaluate pest control and pollinator safety at the macro scale.

We did not receive enough test substance to complete a large plot test with Biological Soil and Foliar. We followed *Lygus* populations in 3 fields we know were treated twice with sulfoxaflor between late June and July. *Lygus* nymph populations remained consistently low in the se plots. However, *Lygus* adults did not appear to be reduced in the treated fields. *Lygus* nymph abundance remained consistently below 2 nymphs per 5 sweeps. Many sweeps yielded no *Lygus* nymphs at all. This documents effective control of *Lygus* with sulfoxaflor. At WASGA I will seek input from growers on what impact they think they are observing on their bees with exposure to sulfoxaflor.

Objective 3. Develop and validate an alkali bee emergence model using cost effective technology. Six alkali bee beds were identified in the Walla Walla Valley for this experiment. A total of 18 HOBO® (Onset Computer Corporation, Bourne MA) loggers were used in this experiment. Using a battery powered hand drill, HOBO® loggers were placed in the soil at depths of 15 cm, 30 cm, and 45 cm on 19-April-2018 at each of the six bee beds studied. HOBO® loggers recorded soil temperatures every 25 minutes from 19 April 2018 until 27 July 2019, approximately 57 recordings per day. At each studied bee bed nine 0.5 m² quadrats were established within a 30.25 m² area of the HOBO® loggers. Quadrats were surveyed twice a week from late May through mid-July by counting the number of emergence holes.

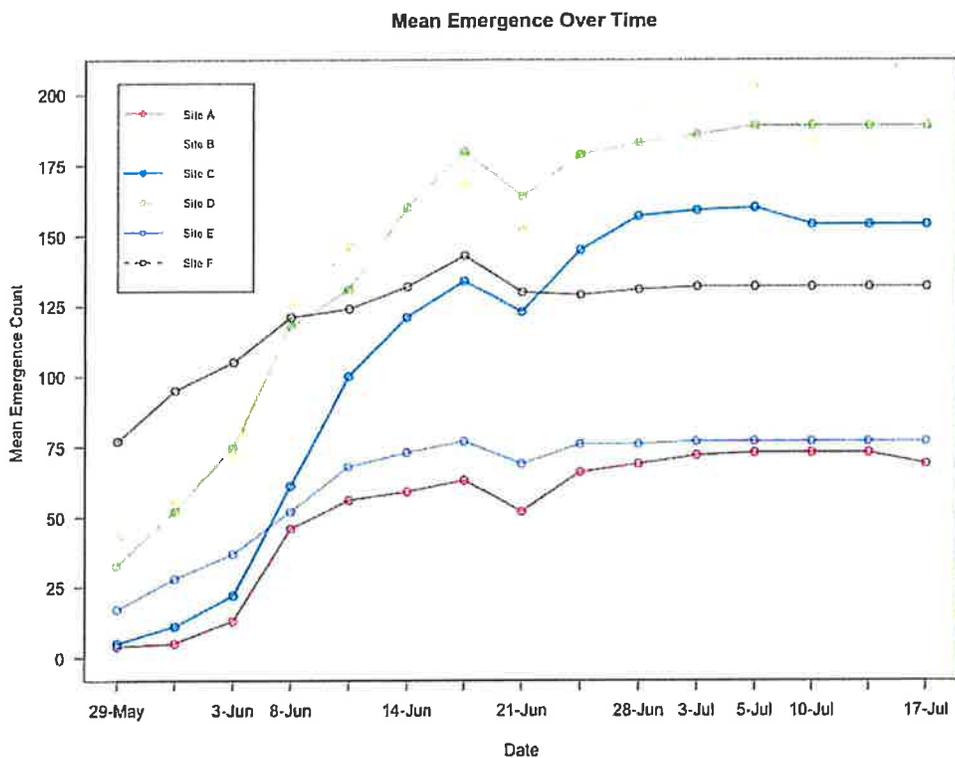
Methodology: Statistical Analysis: At each location, survey data from the nine quadrats were used to calculate mean emergence per day. Onset HOBOWare® (2002) was used to download and import soil temperature data into Microsoft® Office Excel® (2018). Mean soil temperatures at 15cm, 30cm, and 45cm were calculated by day for each location. The mean soil temperature was used to calculate thermal time, $\Delta\tau=(T_i-T_b)$. Average soil temperature (T_i) minus base temperature (T_b) which W.P. Stephen discovered to be 62.6°F. Thermal time was summed to yield cumulative degree-day. In R-Studio Version 1.1.456, a linear mixed effect analysis was used to determine relationship of mean emergence to cumulative degree-days and location. Pearson's correlation analysis was used to determine relationship of mean emergence and cumulative degree-days at each location. Degree-day accumulation data was regressed with mean emergence to quantify the relationship of heat accumulation to date of peak emergence.

At one study site the HOBO® temperature logger at 30 cm experienced water damage and temperature data could not be obtained. Each of the 17 HOBO® loggers recorded 6,008 temperature data points from 19-April until 27-July. Mean emergence data was plotted by date to determine peak emergence date, displayed in Table 1. There was a decrease in mean hole count on 21-June due to a multiday rain event in the Touchet Valley.

Table 1 details the date of peak emergence at each study site.

Location	Peak Date
Site F	18-June
Site D	18-June
Site E	26-June
Site A	28-June
Site B	3-July
Site C	3-July

Figure 1. Alkali bee emergence by study site over time in 2018



Predicted models: We found that cumulative degree-day is a significant predictor of mean number of alkali bee emergence holes (Chi sq_{5,6}= 160.23, p<0.0001). Site location was a significant predictor on mean number of alkali bee emergence holes (Chi sq_{5,6}=220.03 , p<0.0001). Table 2 displays the predicted models generated from analysis of variances at each location.

Table 2: Pearson's correlation (x=Cumulative DD, y=Mean Emergence) and predicted model

<u>Site</u>	<u>Pearson's correlation</u>	<u>Model* cumulative degree days</u>
A	r=0.691 , t28=5.05, p<0.0001	Mean emergence =16.70 + 0.063
B	r=0.753 , t43=7.5, p<0.0001	Mean emergence =63.58 + 0.178
C	r=0.823 , t43=9.51, p<0.0001	Mean emergence = -8.67 + 0.154
D	r=0.782 , t43=8.22 ,p<0.0001	Mean emergence =49.92 + 0.148
E	r=0.706 , t43=6.54 ,p<0.0001	Mean emergence =49.92 + 0.148
F	r=0.575 , t43=4.6, p<0.0001	Mean emergence =99.8 + 0.025

Conclusion: With the six models we can predict peak alkali bee emergence at each of the six studied bee beds. A key improvement of this experiment will be to start surveying bee beds in early May in 2019 to better track initial date of bee emergence. Further model building will incorporate soil temperature depth. These soil temperature-based model will assist alfalfa seed growers by improving their timing of late-spring insecticide sprays while reducing the risk to exposing alkali bees to insecticides.

2018 Report of Work for Alfalfa Pollinator Research Initiative

Project Title:

Year(s) of Study: Summer 2018

Lead Investigator / Affiliation:

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USDA ARS Pollinating Insects Research Unit

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Introduction

For alfalfa seed production, pollinators are a critical partner in the fertilization of flowers and seed set. For alfalfa, the most commonly used bees are two non-*Apis* (non-honey bee) pollinators, the alfalfa leafcutting bee, *Megachile rotundata*, and the alkali bee, *Nomia melanderi*. This proposed research focuses on the alfalfa leafcutting bee (ALCB) and in future research similar experiments will be asked for the alkali bee. In order to have optimal seed set, it is critical that the bees are healthy and have maximal reproduction (i.e., creating high numbers of nest cells requiring pollen and nectar from the alfalfa plants). The act of gathering pollen and nectar ensures that the flowers of the alfalfa are fertilized and seed set. Understanding how crop management practices affect the bee health and ultimate reproduction is critical to ensuring that the bees are performing effectively. The proposed research here fits under two topics of the APRI: III (Safety) and IV (Epidemiology and Disease Management).

In the last 10 years, dramatic losses of pollinators have been reported. The losses are known to have occurred in honey bees and bumble bee populations. For ALCB, the level of bee return has declined to a reported 25-40% in some areas, reflecting a poor reproductive success and possible lower levels of nesting per bee. For honey bees and the other species, several factors (the 4 P's) are contributing to the high yearly losses- pathogens, poor nutrition (pollen availability) and finally pesticide exposure. These same four factors are also observed to affect other pollinator species, with the parasites being pollinator specific. Evidence from experiments reported worldwide indicates that these factors can interact. Some of the factors that may be common across pollination of all crops are adjuvants and pesticides. Besides the active ingredients in pesticides, the "inerts" or adjuvants may be an issue (Mullin, 2015; Mullin et al, 2015; Chen et al., 2018). For the US, the best record of pesticide and adjuvant use is found in the Summary of Pesticide Use Report Data by the California Department of Pesticide Regulation (CDPR) and covers all crops and applications. One type of adjuvants being used is organosilicone spray adjuvants (OSS) that are tank mixed with the pesticides. These compounds are called super spreaders and penetrants and are reported by their manufacturers to increase the efficacy of the pesticides (however, there are very few published studies to support this claim). As reported by the CDPR, the amount of OSS being used has exponentially increased since the mid 2000's, concurrent with the increased losses in honey bee colonies

(Chen et al. 2018). OSSs are applied broadly across all crops listed, including alfalfa and ornamental and lawn/turf. As such, this group of compounds may be a common factor encountered by all pollinators and present in many crop and urban environments.

For OSS, studies indicate that these are toxic to honey bee adults when fed on organosilicone surfactants in 50% sucrose, with oral LC50s for pure commercial trisiloxane surfactants ranging to below 10 ppm, and significant mortality down to 100 ppb (Mullin et al, 2015; Chen et al, 2018). These adjuvants are of concern since trisiloxane surfactants can be detected in 60% of pollen samples from different crops (Chen and Mullin, 2013b). When used in in vitro rearing of honey bee larvae, viral exposure and trisiloxane surfactants synergized to result in highly significant mortality at 10 ppm or lower (Fine, Mullin, Cox-Foster, 2017). In studies in 2016 and 2017, we have found that OSS causes elevated viral levels in adult honey bees and are also toxic to adult bumble bees when fed in sucrose (Cox-Foster unpublished). Trisiloxane surfactants are the primary component of organosilicone adjuvants used in tank mixing and may also be included in formulations of other pesticides. In research trials from 2017, we began to ask how OSS affects ALCB nesting and health. In particular, we wanted to ask how OSS application affected bees via either leaf pieces or the pollen/nectar. To do this, we used two types of plants, one the bees would use for leaf pieces (buckwheat) and another for pollen/nectar (Phacelia). The plants were grown in pots and given to the bees in small cages with nesting blocks provided. Four treatment groups were used, Control (no spray), Leaf Pieces only (Buckwheat sprayed), Pollen/Nectar only (Phacelia sprayed), or both with OSS. Nesting behavior, nesting, and survivorship were monitored; at the end of the experiment, adult females from the cages and their progeny were sampled and frozen for pathogen analyses. Analysis of the nesting success demonstrated that there was a significantly decreased amount of nesting by the adult females when the bees were exposed to either Phacelia or both plants that had been sprayed versus the controls or leaf-piece source treated with OSS. This suggests that OSS somehow affects the health and the reproduction of ALCB. For ALCB, several viral pathogens are known to be present (Singh, 2011; Melathopoulos et al., 2017), including Black Queen Cell Virus that significantly increased in honey bee larvae and adults upon OSS exposure.

Several questions remain on the observed impacts of OSS on the ALCB nesting. How did the OSS affect the pathogen levels in the both the adult bees and their progeny? Did the OSS impact the survivorship of the adult female bees or was the decreased nesting due to disruption of behavior or reproductive physiology? Did the bees have to ingest the pollen/nectar contaminated with OSS or was its impact through contact? How do different levels of OSS exposure affect the bees (i.e., correlate spray applications to exposure and to impacts on the bees)? Answering these questions will allow growers to determine if OSS use can be decreased or replaced by other adjuvants, to improve the health of their bees and increase the nesting success, to maximize the seed set.

Objectives for Years 1 & 2

1. What is the impact of ingestion of OSS on survivorship, reproductive physiology and

- pathogen levels in ALCB?
2. How does OSS affect behavior of the adult ALCB and success as a pollinator?
 3. OSS usage in the field: Does OSS have to be ingested or can the effects come through contact?

Report for Year 1 (of 2) Objectives

Objective 1. Data from lab experiments demonstrates that the OSS is toxic to ALCB adults when ingested at 40 and 100 ppm in sugar water. Additional experiments are needed to ask what the lower limits of the acute toxicity are. Samples have been collected and are awaiting analysis to ask about the impacts upon pathogen levels.

Objective 2. In cage studies, the OSS impacted the number of nests established in the first year. Data has been collected upon the nesting behavior of the bees and is being analyzed.

Objective 3. In cage studies, the levels of pathogens and parasitism by *Mellittobia* was impacted by OSS. The OSS treatments had a significantly increased level of parasitization by *Mellittobia* in the early nest cells. The level of pollen ball was also increased.

It is not clear why the parasitoids were more common in the OSS cages or how they found the bees. We do not think the parasitoids came from the experimental materials themselves. The cages were situated on top of landscape cloth in an area not previously used for alfalfa bee production and the cages were randomized according to treatment. Inside the cages, potted plants in new growing medium and pots were grown. The nests were not previously used and were situated in new sites. The adult bees were paint marked and live bees released. Other cages on alfalfa plots were nearby and the ALCB nests in those cages did not have noticeable parasitization. Experiments in Year 2 will ask if the attractiveness of the nests of OSS bees is somehow altered to cause the increased parasitization.

Experiments are continuing to ask about the level of pathogens in the OSS exposed bees.

Conclusion

Research thus far has been informative and will continue into 2019 and through 2020 to ask how OSS impacts the health of ALCB and if actual field use of the adjuvants is associated with declined bee nesting and pollination.

Chalkbrood control potential of hypochlorous acid in managed alfalfa leafcutting bee populations

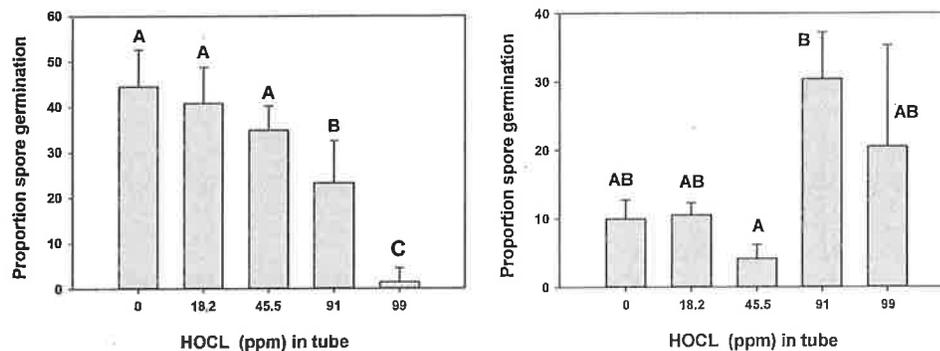
Ellen Klinger and Diana Cox-Foster, USDA-ARS Pollinating Insect Research Unit, Logan, UT

We assessed the potential of Hypochlorous Acid (HOCL) as a safe sterilant for chalkbrood contamination of alfalfa leafcutting bee cocoons and nesting material. Hypochlorous acid (HOCL) is a form of free chlorine that has high antimicrobial activity, very low environmental persistence and a potential low degree of harm for bees and humans. The product has been patented and produced by a commercial company and approved by FDA for microbial control on skin. The company hopes to develop the product for sterilization of medical suites and equipment.

For initial trial, we isolated spores from *Ascosphaera aggregata*, the species of fungus that causes chalkbrood disease in alfalfa leafcutting bees (ALCB) and tested the efficacy of various concentrations of HOCL on the survival of the spores. While testing spores from the fungus that caused chalkbrood in the Blue orchard bee, we saw less spores surviving with increasing HOCL concentrations (Fig. 1). However, with the ALCB chalkbrood species, we surprisingly did not see the same pattern (Fig. 2). We are currently unsure why spore survival seems to be stimulated by HOCL, but it does not seem to be highly effective for spore kill with *Ascosphaera aggregata* in the manner used.

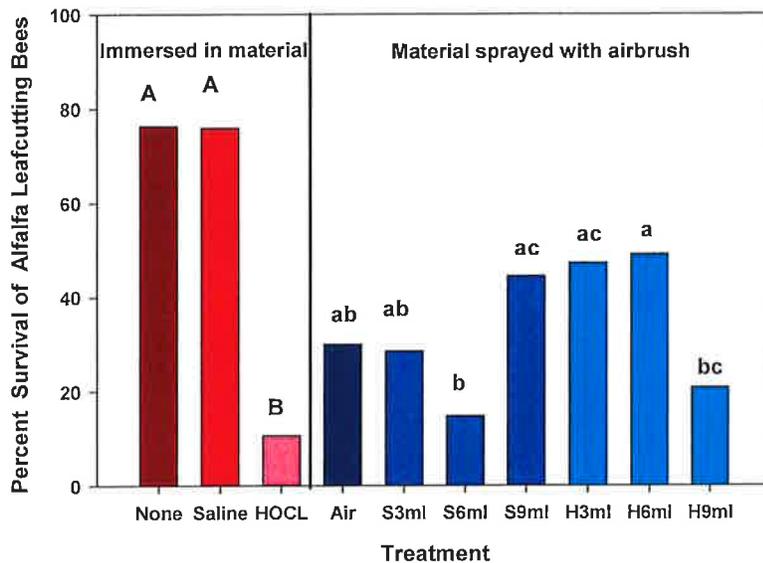
Figure 1(Left). Proportion germinated (live) fungal spores when treated with various concentrations of HOCL- Blue orchard bee chalkbrood species, *Ascosphaera torchioi*

Figure 2(Right). Proportion germinated (live) fungal spores when treated with various concentrations of HOCL- ALCB chalkbrood species, *Ascosphaera aggregata*



*Statistical test- Generalized linear model, significance of HOCL proportion $F=4.80$, $p=0.0202$, Tukeys multiple comparison between groups

Artificially growing spores in a laboratory may not present a true depiction of what may happen biologically, so we applied it in a more field based manner. We tested if HOCL was toxic to ALCB cocoons with an airbrush application of material, or via immersion. We used saline as a blank. When material (either saline or HOCL) was applied to the cocoons via airbrush, there was no significant reduction of ALCB emergence after incubation. However, immersing the cocoons in HOCL did appear toxic to the bees (Figure 3).



*Statistical test- Generalized linear model, significance of HOCL proportion $F=14.46$, $p<0.001$, Tukeys multiple comparison between groups

We also conducted a field test where we applied chalkbrood spores to the outer surface of ALCB cocoons, treated these contaminated cocoons with HOCL via airbrush and released the bees inside of small cages with plenty of alfalfa forage. We let the bees nest for several weeks, then brought the nests into the lab and x rayed them on a weekly basis. We used the x rays to score the health of the bees. HOCL treatment did not have a significant effect on the proportion of healthy or chalkbrood contaminated bees. Treatment did have a close to significant effect ($p=0.06$) on pollen ball, reducing pollen ball from 32.7% of nests to 19.5% of nests. This raises the interesting prospect that another microbe could have been present and killed by the treatment; this needs to be addressed in future experiments.

*Statistical test- Logistic regression, with health category of interest as event

Table 1.

Health condition	Control	HOCL
Healthy	59.6%	66.0%
Chalkbrood	1.4%	2.7%
Pollen Ball	32.7%	19.5%

So, while HOCL may show promise for some chalkbrood fungal species, it does not seem to be highly effective against the species causing chalkbrood in the alfalfa leafcutter bee. While there may be something specific about this disinfectant material and potentially other methods in which it could be used, there may be other, non-toxic alternatives worth testing for chalkbrood control in ALCB. In year 2 of our project we propose testing the HOCL at other concentrations and in combination with other anti-

microbials. We also plan to test the chemical again to see if the reduction in pollen ball is reproducible; if it can be reproduced, we will conduct molecular tests to see if there are changes in other microbes associated with the bees and cocoons.

Study of life cycles of *Melittobia* and *Pteromalus* in populations of *Megachile rotundata* in summer, fall, and winter (Year 1 Report)

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Introduction

Commercial populations of the alfalfa leafcutting bee (ALCB; *Megachile rotundata*) from the western United States often suffer from high rates of parasitism by various wasp species. In particular, *Melittobia* has been found to be an egregious pest of ALCB populations in Utah and Idaho, eliciting a higher than usual level of vigilance and anxiety. Other common and persistent pests of ALCBs are *Pteromalus venustus*, *Tetrastichus* sp., *Monodontomerus obscurus* and *Sapyga pumilla*. The most common of these, which are pests during both summer incubation and fall storages periods, is *P. venustus*.

In 2018, Utah State University graduate student Alan Anderson worked to meet some of the four proposed objectives at PIRU as part of a Master's Thesis. He acquired new information about the overwintering of *Melittobia*. In addition, he has compared the timing of adult emergence timing of this wasp with that of *P. venustus*.

APRI Proposal Objectives, Years 1 & 2

1. Search data collected from Utah field studies in 2011 and 2014 for determining timing and conditions for infestation by parasites and parasitoids in commercial shelters during ALCB nesting season.
2. Determine prewintering and overwintering conditions for *Melittobia* and *Pteromalus* using ALCB prepupae as hosts.
3. Determine *Melittobia* and *Pteromalus* life stages that overwinter with managed ALCBs.
4. Monitor *Melittobia* and *Pteromalus* life cycles during bee incubation to determine if there is overlap of parasitoid emergence and if a common protocol can be employed to monitor and curtail infestations.

Report for Year 1 (of 2) Objectives

Objective 1. Data from field studies in 2011 and 2014 ALCB populations have been organized and are in the process of being cleaned and sorted for further analyses of incidence of parasites over time. Findings may be more limited than first projected because during these studies, ALCB nests were removed weekly from fields (and kept near PIRU at ambient temperatures). Thus, while bees were still on the farm, cells contained eggs and larvae, but did not contain the ALCB life stage (prepupa) that would be attacked by *Melittobia* and *P. venustus*. If prepupal parasitoids attacked ALCB prepupae, the attacks would have occurred later at PIRU.

Objectives 2 & 3. In October 2017, ALCB prepupae were exposed to *Melittobia* and *P. venustus* for 3, 6 or 9 days. Half of the cells were kept at 86°F incubator, and half remained at ambient temperature in an unheated shop on a table top during the exposure periods [exposure period (3 X 60) by temp type (2): n = 360 cells]. Then all prepupal cells were placed in cold storage (40°F). Cells were incubated at 86°F on 01 June 2018 and, after emergence of live adult bees and wasps, were inspected to determine remaining contents.

Figure 1a: Three Days of Exposure

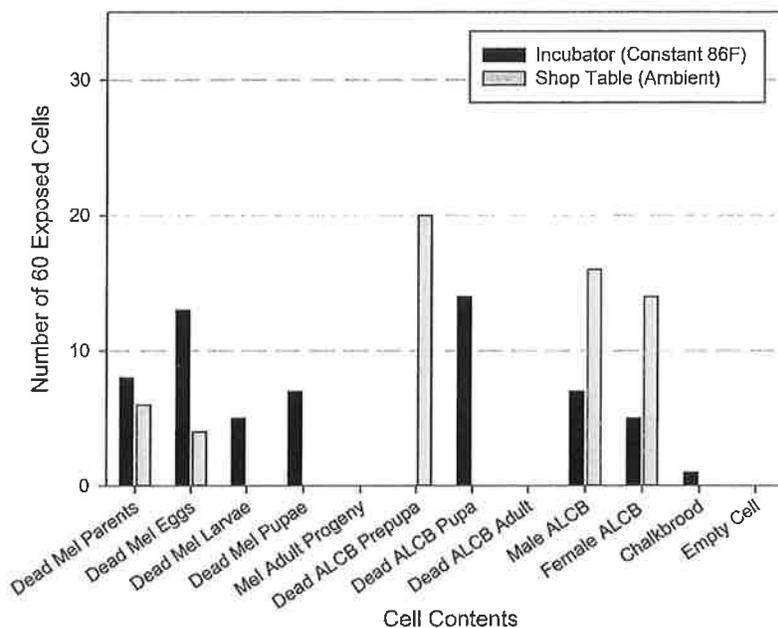
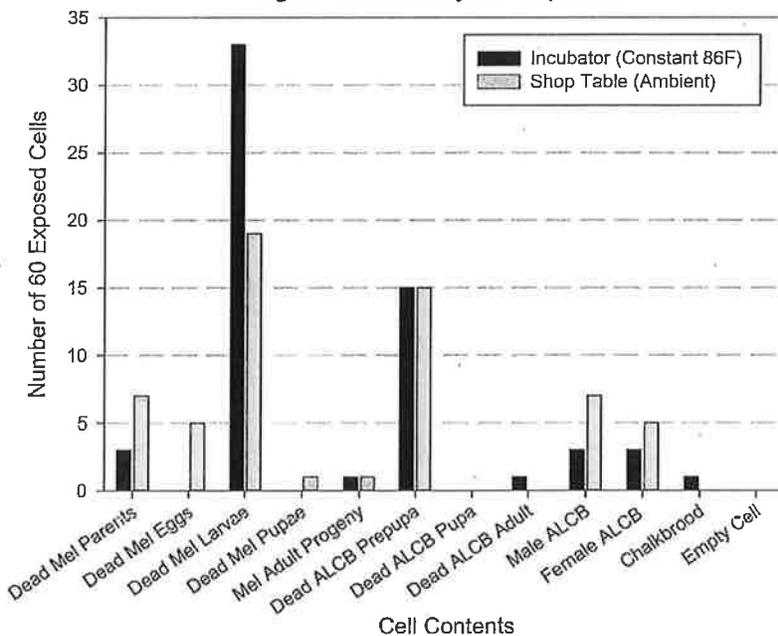


Figure 1b: Six Days of Exposure



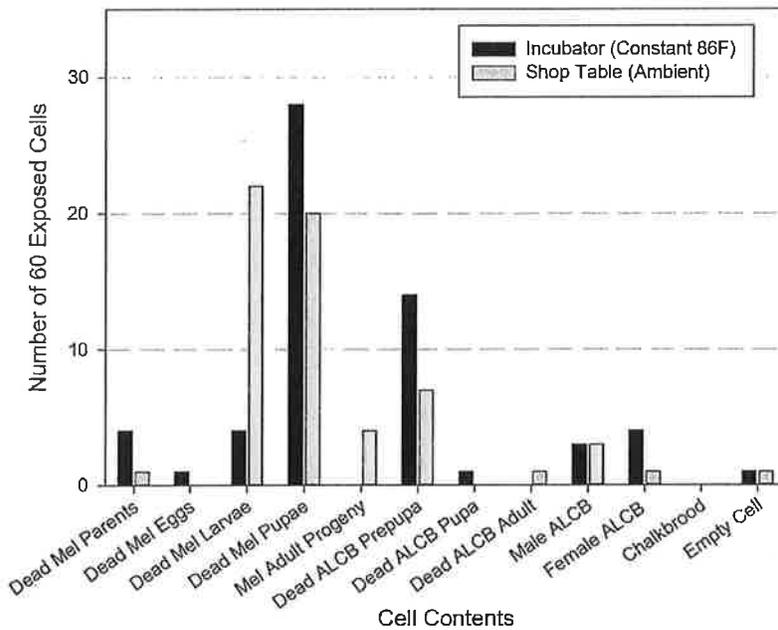
prepupae were parasitized by *Melittobia*, but the wasps did not live past larval stages (Fig. 1b). Many ALCB prepupae also died under these conditions. Of the few ALCB adults that emerged, most were from the ambient exposure treatment (Fig. 1b).

After 9 days of exposure at constant temperature, almost half of all ALCB prepupae supported *Melittobia* to the pupal stage, with fewer cells harboring pupal parasites if kept at ambient (Fig. 1c). For cells held at ambient temperature, more wasps died as larvae compared to

Results showed that regardless of the exposure duration and whether *Melittobia* had access to ALCB prepupae at fluctuating ambient or at constant temperatures, only a few *Melittobia* progeny survived to reach the (dead) adult stage; even fewer emerged as live adults (Figs. 1a-c). Also, some of the parental wasps were found inside the cells (with dead ALCB prepupae) after wintering. For only 3 days of exposure at constant temperature, the *Melittobia* progeny lived through larval stages, and some reached the pupal stage (Fig. 1a). If left at ambient on a shop table, the *Melittobia* mothers laid eggs, but ambient temperatures prevented eggs from developing further before going into cold storage (Fig. 1a). Many ALCB cells were unparasitized when left at ambient for only 3 days, and ALCB adults emerged from the cells (although many also died as prepupae from unknown causes) (Fig. 1a.)

After 6 days of exposure, many ALCB

Figure 1c: Nine Days of Exposure



those at constant temperature. At 9 days of parasite exposure prior to cold storage, the least number of ALCBs survived to adulthood (Fig. 1c) compared to shorter exposure times.

Based on these findings, we tentatively conclude that the *Melittobia* life stage that successfully survives the winter is the prepupal stage. We are continuing this line of research in 2018-2019.

Objective 4. From a local stock of bees at PIRU, cells diagnosed from x-ray analysis as “parasitized” were monitored for emergence of all adult *Melittobia*, *P. venustus* and *Tetrastichus* sp. (n = 50-60 cells each). For each species, the emergence of the first wasp was recorded. Also the duration of adult emergence (day of first wasp to emerge until day of last wasp to emerge) was determined.

Results show that most *Melittobia* begin to emerge 2-3 days ahead of *P. venustus* and 4 days ahead of *Tetrastichus* sp. (Fig. 2). The duration of emergence for *Melittobia* is long, continuing out to about 9 days. Emergence timing and duration of *P. venustus* and *Tetrastichus* sp. are similar, and the duration is brief (3-4 days) (Fig. 3).

Figure 2: First Day of Wasp Emergence

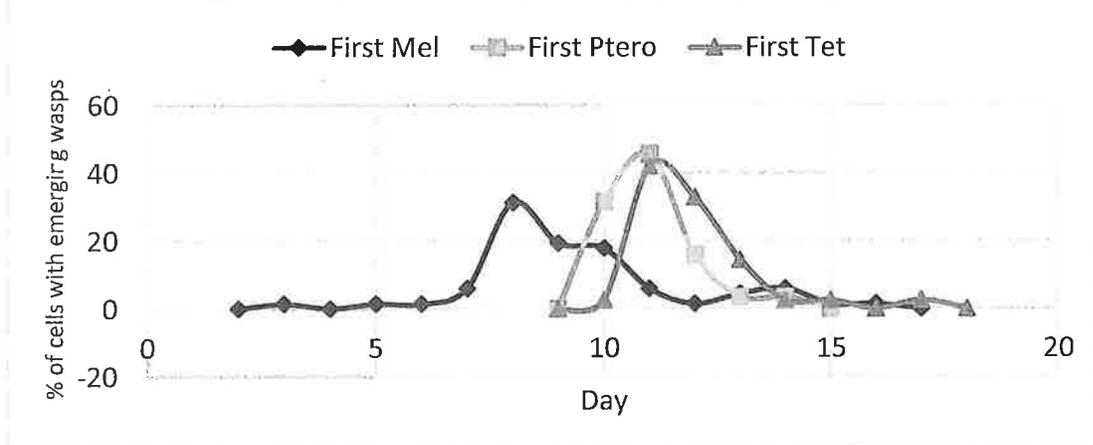
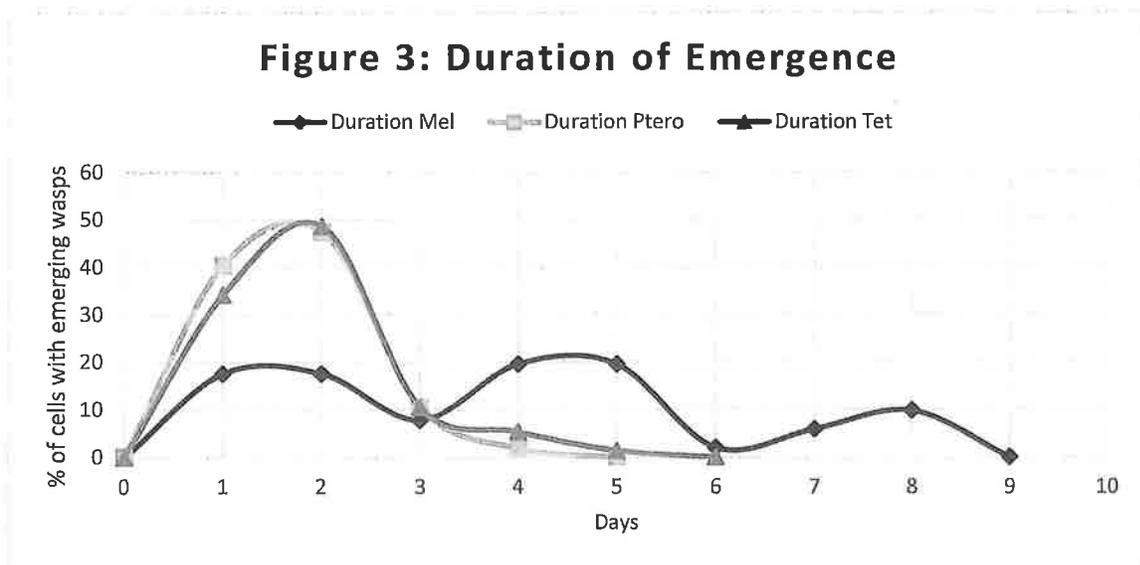


Figure 3: Duration of Emergence



Conclusion

Research thus far has been successful, and results will help design projects that continue into 2019 and through 2020. More life history traits of the parasitic wasps will be revealed and may afford the opportunity to develop traps or monitoring devices for these pests.

A modified seedling phenotypic assay to identify glyphosate resistance in different alfalfa varieties

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Abstract

To determine the general applicability of a seedling phenotypic assay, previously developed by Boyle and colleagues to distinguish between conventional and glyphosate-resistant seedlings, we extended the assay to four conventional and four glyphosate-resistant alfalfa varieties. We also tested the assay under more generic laboratory conditions, using petri dishes placed in an incubator. Finally, we tested and confirmed that a 16h light: 8h dark regime, 80 ppm glyphosate concentration and a 14-day germination period, represented the best conditions to separate conventional and glyphosate-resistant seedlings using the phenotypic assay. Contrary to Boyle and colleagues, we did not observe the presence of root hair in all glyphosate-resistant seedlings exposed to 80 ppm glyphosate, although none of the conventional seedlings had root hair. Variation in the percentage of glyphosate-resistant seedlings with root hair was detected among varieties in both the control and the 80 ppm glyphosate treatment. We identified the presence of secondary roots as an additional phenotypic trait useful for this phenotypic assay. Variation among varieties was also detected for percent seedlings with secondary roots. Such variation will affect the sensitivity of the phenotypic seedling assay among varieties. We therefore do not expect the presence/absence of root hair and secondary roots between glyphosate-resistant and conventional seedlings to be 100% accurate when extended to different varieties. We propose a methodology that combines the phenotypic seedling assay with the use of the RUR test strips to detect glyphosate-resistant seedlings. This methodology can be applied to different alfalfa varieties under more generic laboratory conditions and will be useful to detect gene flow events and AP presence in alfalfa seed-production fields.

Introduction

Several varieties of genetically engineered (GE) alfalfa are commercially available in the United States. The first GE trait introduced in alfalfa was glyphosate resistance, which facilitates weed control in “Roundup Ready” (RR) alfalfa fields using the broad-spectrum herbicide Roundup (Van Deynze et al. 2004). More recently, low lignin alfalfa has been developed and the lower lignin content makes these varieties more easily digestible by cows (Undersander et al. 2009). As with many agricultural crops, alfalfa seed is produced for a variety of markets, including the biotechnology, conventional, organic, and export markets. Within the organic and export markets, thresholds for adventitious presence (AP), or the unintentional introduction of GE traits in seed, are very low. Despite the use of grower opportunity zones and non-GE seed production areas, best management practices to minimize AP (NAFA 2015), gene flow and adventitious presence are still occurring (Kesoju and Greene 2017). Therefore better methods are still needed

to effectively limit AP while allowing for successful coexistence of GE and non-GE alfalfa (Mueller 2004, Putnam et al. 2016).

Studies of gene flow from glyphosate-resistant to non-GE alfalfa fields have identified gene flow events via the presence of glyphosate resistance in seeds collected from non-GE fields. The current method of detection for glyphosate resistance in alfalfa seeds or seedlings is the use of lateral flow immunoassay AgraStrip RUR Seed & Leaf test strips (Romer Labs Inc., Newark, DE). While very effective, this method is costly for large gene flow experiments and therefore the need to develop a more cost-effective method of detection of glyphosate resistance in alfalfa seeds. To address this need, Boyle et al. (2016) developed a seedling germination assay. They germinated alfalfa seeds in an 80 ppm glyphosate solution and looked for phenotypic traits of seedlings associated with glyphosate resistance. They identified longer, narrower roots and the presence of root hairs in 14 day-old seedlings as phenotypic traits that could distinguish glyphosate-resistant from conventional or non-GE seedlings. In their study, all glyphosate-resistant seedlings had visible root hairs whereas all conventional seedlings did not. Glyphosate resistance identified using phenotypic traits was confirmed using the AgraStrip RUR test strips (Romer Labs Inc., Newark, DE).

Boyle et al. (2016) examined one variety of glyphosate-resistant alfalfa (Monsanto DKA41-18RR) and one variety of conventional alfalfa (Pioneer WAPH-1 lot no. Wy8932). Before using their phenotypic assay in an experiment linking pollinator behavior to gene flow (Brunet, 2018), we tested its general applicability using different alfalfa varieties. We also examined the effectiveness of the assay under more generic laboratory conditions. Boyle et al. (2016) germinated seeds on germination paper put on perforated germination trays fitted for water curtain germinators while we tested germinating seeds on filter paper in petri dishes placed in an incubator. Finally, Boyle et al. (2016) let seeds germinate at 20° C with a 16 h light: 8 h dark cycle using an 80 ppm glyphosate concentration, allowing the seedlings to grow for 14 days. We determined whether seeds could be grown in the dark, and tested different glyphosate concentrations and a growth period of less than 14 days. In the current study, we determined how more generic laboratory procedures, light regime, glyphosate concentration and the period of seedling growth affected the results using eight varieties of alfalfa, four conventional and four glyphosate-resistant varieties. A generally applicable seedling phenotypic assay would benefit gene flow studies and such studies could pinpoint methods to further reduce gene flow and thus facilitate the coexistence of the different alfalfa seed-production markets.

Materials and Methods

General procedures

In all experiments described below, seeds were placed in petri dishes lined with filter paper (Fisher Scientific, Pittsburgh, PA) and put in a generic incubator chamber (model I-30VL, Percival Scientific Inc., Perry, IA) at 20°C. Following Boyle et al. (2016), distilled water or a low concentration glyphosate solution was used to keep the seeds moist on the initial set-up and three days later, while a Plant Preservative Mixture (PPM™, Caisson Laboratories, Inc., Smithfield, UT) solution of 1ml PPM: 1 liter distilled water was used as needed for the remaining germination period.

Light, glyphosate concentration and growth period

In this experiment, we tested the impact of light regime, glyphosate concentration and seedling germination period on the presence of root hair in one glyphosate-resistant (DKA44-16RR) and one conventional (Legacy455HR) alfalfa variety. We grew seeds either in the dark or under a 16h light: 8h dark regime and within each treatment we tested a control (distilled water) and two glyphosate concentrations, 80 ppm and 200 ppm. We set up separate experiments for each of two time periods, 7 and 14 days, because prior experiments had shown that manipulation of seedlings during the germination period can result in the dehydration of the root hairs, which are a lateral extension of a single epidermal cell. A petri dish with 5 seeds was the replicate in this experiment and there was a minimum of three replicates per treatment. The experiment tested two light treatments, three glyphosate concentrations (one being the control), two varieties and two germination durations for a total of (3 petri dishes (rep) * 2 light * 3 conc * 2 var * 2 periods) 72 petri dishes.

At the end of the 7-day germination treatment, we examined each seedling individually for the presence of root hair. During this assessment, we noticed the development of secondary roots in several seedlings in both conventional and glyphosate-resistant varieties (Fig. 1). We thus decided to examine both the presence of root hair and of secondary roots in the 14-day germination treatment. After examining each seedling for the presence of root hair and secondary roots, we calculated the percentage of seedlings with root hair and the percentage with secondary roots in each petri dish. We compiled the results and established the experimental conditions that permitted the best separation between conventional and glyphosate-resistant seedlings.

Alfalfa variety and category

We tested four conventional (Legacy455HR, F-SG403LR, Pioneer55H94, and Toughbox) and four glyphosate-resistant (DKA44-16RR, DKA43-22RR, 428RR, and Powerhouse RR) alfalfa varieties. We grew seedlings under a 16 h light: 8 h dark light regime, using a 14 day germination period and an 80 ppm glyphosate solution. These conditions best separated the two categories, glyphosate-resistant and conventional seedlings, as confirmed in our previous experiment. After 14 days, we measured seed germination, percentage of seedlings with root hair, percentage of seedlings with secondary roots, and root length in a control (distilled water) and an 80 ppm glyphosate treatment. For root length, a picture of each petri dish was taken on day 14 and the length of the radicle was measured for each seedling using ImageJ software (Abramoff et al. 2004). We tested two seed densities in the experiment, either 5 or 10 seeds per petri dish. A petri dish was the replicate in the experiment.

We first used a two-way analysis of variance to determine the impact of seed density and alfalfa variety and their interaction on each dependent variable (seed germination, % root hair, % secondary root and root length) in the glyphosate treatment. The results indicated that using 10 seeds per petri dish was preferable and further analyses were done using only the 10 seeds per petri dish data. One way analyses of variance determined the impact of alfalfa variety on each dependent variable, first in the glyphosate treatment and then in the control. A contrast statement compared the two categories, glyphosate-resistant and conventional. We then combined the two treatments and used a two-way analysis of variance to determine the impact of variety and treatment and their interaction on each dependent variable.

Analyses of the glyphosate treatment highlighted differences among varieties and between the two categories and helped identify the phenotypic traits that best separated conventional and glyphosate-resistant seedlings. Analyses of the control treatment established the baseline variation among varieties and between categories for the various dependent variables examined in this study. Finally, comparing the control and glyphosate treatments helped determine how the glyphosate treatment affected the various dependent variables relative to the control for both conventional and glyphosate-resistant varieties.



Figure 1. Conventional (left) and glyphosate-resistant (Roundup Ready) (right) alfalfa seedlings germinated under control (upper panels) and glyphosate (bottom panels) treatment. Glyphosate resistance of seedlings (bottom right) is indicated by longer and thinner roots and secondary roots development (arrows) relative to conventional (bottom left). Scale bars = 1cm.

Results

Light, glyphosate concentration and growth period

After 7 days in the dark, some conventional seedlings had root hair at both glyphosate concentrations (Fig. 2). After 14 days, we observed no seedlings with root hairs for any of the varieties, conventional or glyphosate-resistant, under either glyphosate concentration (data not shown). We therefore eliminated the dark treatment from further analyses and concluded that seedlings should be grown under the 16 h light: 8 h dark regime.

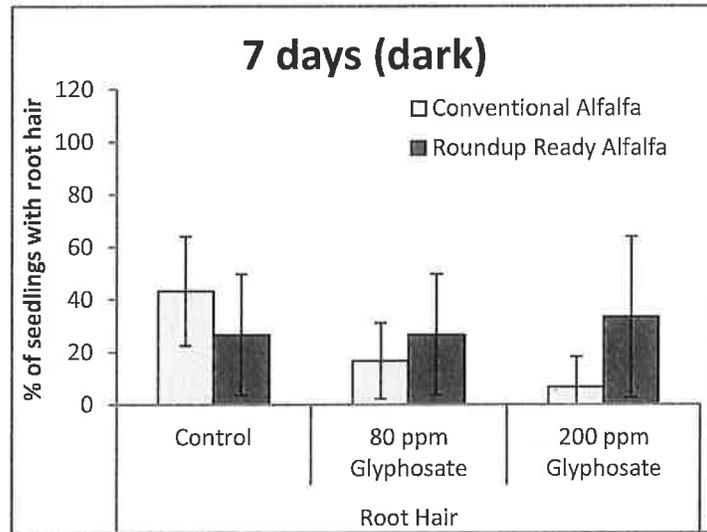


Figure 2. The percentage of seedlings with root hairs after 7 days for seedlings grown in the dark. Control group was germinated in distilled water and glyphosate groups were exposed to herbicide on days one and three of the experiment. Error bars represent standard deviation.

When examining the 16 h light: 8 h dark treatment after 7 days, none of the conventional seedlings had root hair at the 200 ppm glyphosate concentration but (mean \pm STD) 19.4% \pm 17.3 of the conventional seedlings had root hair at the 80 ppm glyphosate concentration (Fig. 3a). However, after 14 days, none of the conventional seedlings had root hair at either glyphosate concentration (Fig. 3b). Moreover, the proportion of glyphosate-resistant seedlings with root hair was greater at the 80 ppm glyphosate concentration. After 14 days, 69.4% \pm 33.7 of the glyphosate-resistant seedlings had root hairs at the 80 ppm glyphosate concentration relative to 38.3% \pm 37.5 at the 200 ppm glyphosate concentration (Fig. 3b). In the control treatment, 75.0% \pm 25.0 of glyphosate-resistant and 31.7% \pm 16.1 of conventional seedlings had root hair after 14 days (Fig. 3b). The glyphosate treatment increased the contrast in the percentage of seedlings with root hair between the glyphosate-resistant and conventional seedlings. We therefore conclude that a growth period of 14 days and a glyphosate concentration of 80 ppm are better conditions to distinguish between conventional and glyphosate-resistant seedlings based on the presence or absence of root hair.

None of the conventional seedlings had secondary roots at either glyphosate concentration (Fig. 4). A greater proportion of the glyphosate-resistant plants exhibited secondary roots at the 80 ppm (65.7% \pm 15.0%) relative to the 200 ppm glyphosate concentration (16.7% \pm 14.4%). In

contrast, in the control group, secondary roots were found in $91.7\% \pm 14.4$ of both the glyphosate-resistant and the conventional seedlings (Fig. 4). Data on secondary roots confirms the pattern found for root hair where a 14-day period and an 80 ppm glyphosate solution represent the best conditions to distinguish conventional from glyphosate-resistant seedlings.

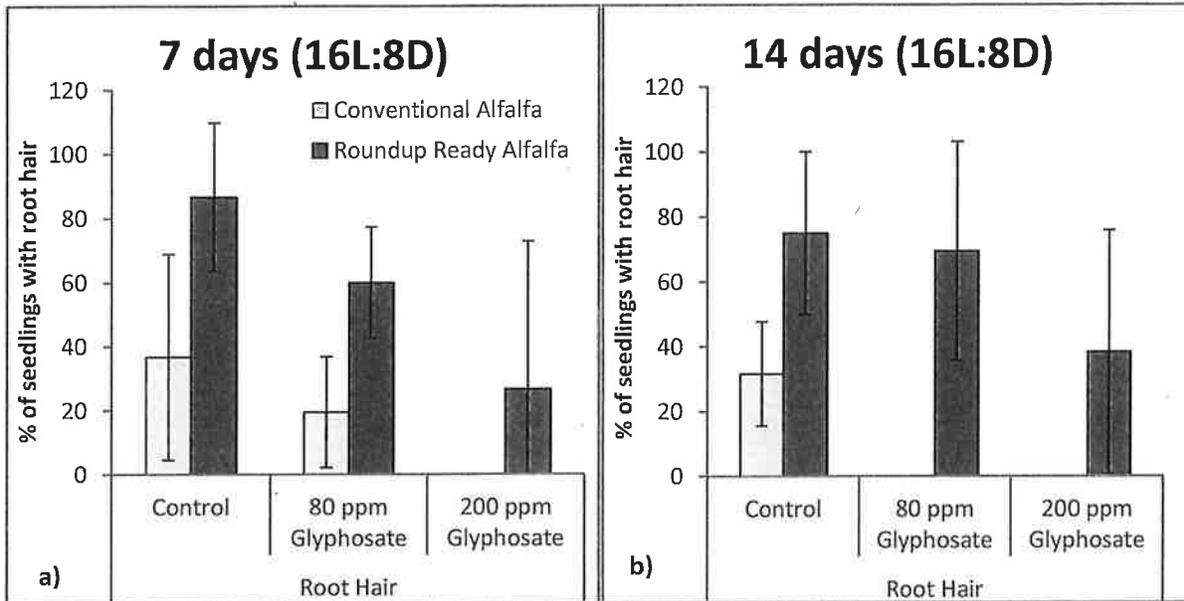


Figure 3. The proportion of seedlings with root hair after a) 7 or b) 14 days grown in a 16h light: 8h dark regime under different glyphosate concentrations. Control group was germinated in distilled water and glyphosate groups were exposed to herbicide on days one and three of the experiment. Error bars represent standard deviation.

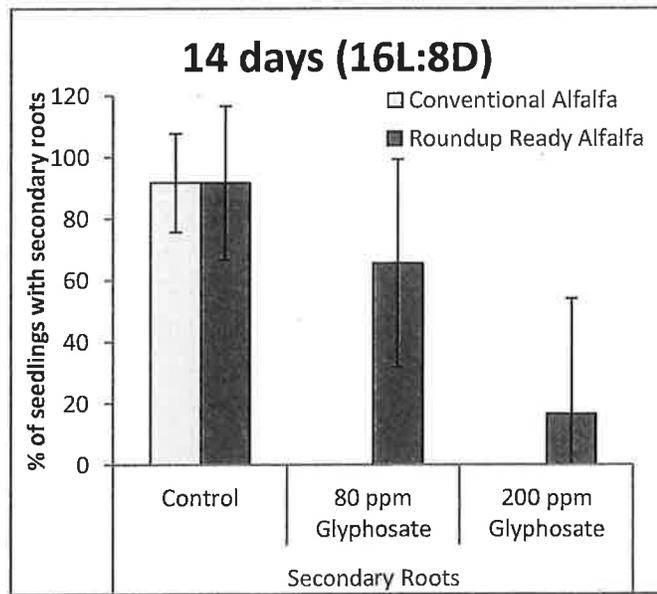


Figure 4. The percentage of alfalfa seedlings exhibiting secondary roots after 14 days grown in a 16h light: 8h dark regime. Control group was germinated in distilled water and glyphosate groups were exposed to herbicide on days one and three of the experiment. Error bars represent standard deviation.

Alfalfa variety and category

Glyphosate treatment

We did not find differences in seed germination ($F_{1,32} = 0.74$, $P = 0.39$) or percentage of secondary roots ($F_{1,32} = 0.01$, $P = 0.92$) between the two seed densities. However, the percentage of root hair ($F_{1,32} = 14.45$, $P = 0.0006$) and root length ($F_{1,32} = 54.80$, $P < 0.0001$) varied with seed density. For root hair, there was a statistically significant interaction between seed density and variety ($F_{7,32} = 6.10$, $P = 0.0001$). We determined that seedlings of one of the glyphosate-resistant varieties had no root hair at a density of 5 seeds per petri dish but had root hair at a density of 10 seeds per petri dish. When this variety was removed from the analyses, density no longer affected the percentage of root hair ($F_{1,28} = 1.73$, $P = 0.20$) and there was no longer an interaction between variety and density ($F_{6,28} = 0.77$, $P = 0.60$). However, root length was still affected by density ($F_{1,28} = 17.65$, $P = 0.002$) with longer roots at higher seed density. Moreover, there was still an interaction between variety and density on their impact on root length and two of the glyphosate-resistant varieties, 428RR and Powerhouse RR, had significantly longer roots at higher seed densities ($t_{1,28} = 2.99$, $P = 0.006$ for 428RR and $t = 5.3$, $P < 0.001$ for Powerhouse RR). We therefore decided to use only the 10 seeds per petri dish treatment in the next analyses and recommend doing these tests using 10 seeds per petri dish.

At the end of the 14-day period, seed germination did not vary among varieties ($F_{7,16} = 1.23$, $P = 0.34$) and we observed no statistically significant difference in seed germination between categories, conventional (mean \pm STD) ($85\% \pm 13.8$) and glyphosate-resistant varieties ($80.8\% \pm 12.4$) ($F_{1,16} = 0.31$, $P = 0.58$). Seed germination ranged between 76.7 and 93.3% among varieties (Table 1).

Table 1. Germination rate, percentage of seedlings with root hair, percentage of seedlings with secondary roots, and root length for conventional and glyphosate-resistant alfalfa varieties grown in an 80 ppm glyphosate treatment for 14 days. Data are mean \pm standard deviation. Statistically significant differences between varieties for each metric (i.e. within columns) are noted with different letters.

Seed variety	Germination Rate	% with Root Hair	% with Secondary Roots	Root Length
Conventional Alfalfa				
Legacy455HR	90.0% \pm 0.0 ^a	0.0% \pm 0.0 ^a	0.0% \pm 0.0 ^a	12.9mm \pm 1.1 ^a
F-SG403LR	93.3% \pm 5.8 ^a	0.0% \pm 0.0 ^a	0.0% \pm 0.0 ^a	10.9mm \pm 0.9 ^a
Pioneer55H94	76.7% \pm 15.3 ^a	0.0% \pm 0.0 ^a	0.0% \pm 0.0 ^a	11.8mm \pm 0.3 ^a
Toughbox	80.0% \pm 17.3 ^a	0.0% \pm 0.0 ^a	0.0% \pm 0.0 ^a	10.6mm \pm 1.1 ^a
Glyphosate-resistant Alfalfa				
DKA44-16RR	80.0% \pm 17.3 ^a	75.7% \pm 8.7 ^d	86.7% \pm 23.1 ^b	36.0mm \pm 3.6 ^b
PowerhouseRR	90.0% \pm 10.0 ^a	53.9% \pm 25.1 ^{bc}	67.6% \pm 15.3 ^c	26.7mm \pm 2.1 ^c
DKA43-22RR	76.7% \pm 15.3 ^a	40.7% \pm 21.0 ^b	78.2% \pm 13.9 ^{bc}	22.8mm \pm 2.0 ^d
428RR	76.7% \pm 15.3 ^a	68.1% \pm 18.8 ^{cd}	78.7% \pm 4.2 ^{bc}	30.6mm \pm 1.8 ^e

The percentage of root hair differed among varieties ($F_{7,16} = 26.8$, $P < 0.0001$) and between categories ($F_{1,16} = 51.2$, $P < 0.0001$). In fact, no conventional seedlings exposed to glyphosate had root hair (Table 1). The average proportion of seedlings with root hair for glyphosate-resistant seedlings exposed to glyphosate was $59.6\% \pm 21.6$ and ranged between 40.7% and 75.7% among varieties (Table 1). Of the glyphosate-resistant varieties, the proportion of root hair differed significantly between varieties 428RR and DKA43-22RR ($t = 2.99$, $P = 0.0086$), varieties DKA43-22RR and DKA44-16RR ($t = 3.83$, $P = 0.0015$) and varieties DKA44-16RR and Powerhouse RR ($t = 2.39$, $P = 0.03$) (Table 1). For secondary roots, we observed significant differences among varieties ($F_{7,16} = 64.5$, $P < 0.0001$) and significant differences between categories ($F_{1,16} = 142.49$, $P < 0.0001$). None of the conventional seedlings exhibited secondary roots but $77.8\% \pm 15.1$ of the glyphosate-resistant seedlings had secondary roots. The percentage of secondary roots among glyphosate-resistant varieties ranged between 67.6% and 86.7% and was significantly different between all glyphosate-resistant varieties and the conventional varieties (Table 1). Only two of the glyphosate-resistant varieties, DKA44-16RR and PowerhouseRR differed from each other ($t = 2.59$, $P = 0.02$) (Table 1).

Varieties differed in root length ($F_{7,16} = 129.25$, $P < 0.0001$) and conventional varieties had shorter roots (11.5 ± 1.2 mm) relative to glyphosate-resistant varieties (29.0 ± 5.6 mm) ($F_{1,16} = 232.07$, $P < 0.0001$). In fact, each glyphosate-resistant variety had significantly longer roots than any of the conventional varieties (Table 1).

Control

In the control, seed germination did not differ significantly among varieties although the effect was borderline ($F_{7,16} = 2.47$, $P = 0.063$). We observed no difference in seed germination between categories, conventional ($79.2\% \pm 15.2$) and glyphosate-resistant varieties ($85\% \pm 5.8$) ($F_{1,16} = 0.03$, $P = 0.87$). Seed germination ranged between 63.3 and 100% among varieties (Table 2).

Table 2. Germination rate, percentage of seedlings with root hair, percentage of seedlings with secondary roots, and root length for conventional and glyphosate-resistant alfalfa varieties grown in a distilled water control for 14 days. Data are mean \pm standard deviation. Statistically significant differences between varieties for each metric (i.e. within columns) are noted with different letters.

Seed variety	Germination Rate	% with Root Hair	% with Secondary Roots	Root Length
Conventional alfalfa				
Legacy455HR	73.3% \pm 15.3 ^a	100.0% \pm 0.0 ^c	100.0% \pm 0.0 ^a	52.7mm \pm 4.1 ^{bd}
F-SG403LR	76.7% \pm 15.3 ^a	77.3% \pm 10.4 ^{ab}	100.0% \pm 0.0 ^a	38.6mm \pm 10.0 ^b
Pioneer55H94	63.3% \pm 15.3 ^a	67.5% \pm 30.3 ^a	100.0% \pm 0.0 ^a	44.8mm \pm 6.2 ^{bd}
Toughbox	100% \pm 0.0 ^a	100.0% \pm 0.0 ^c	100.0% \pm 0.0 ^a	50.3mm \pm 3.1 ^{bd}
Glyphosate-resistant alfalfa				
DKA44-16RR	80.0% \pm 20.0 ^a	100.0% \pm 0.0 ^c	100.0% \pm 0.0 ^a	63.7mm \pm 22.5 ^{ad}
PowerhouseRR	90.0% \pm 10.0 ^a	100.0% \pm 0.0 ^c	100.0% \pm 0.0 ^a	80.3mm \pm 25.8 ^{ac}
DKA43-22RR	80.0% \pm 10.0 ^a	100.0% \pm 0.0 ^c	92.6% \pm 12.8 ^{ab}	87.7mm \pm 9.1 ^c
428RR	90.0% \pm 17.3 ^a	87.1% \pm 14.5 ^{bc}	83.8% \pm 14.7 ^b	64.3mm \pm 19.6 ^{ad}

Varieties had a statistically different percentage of seedlings with root hair ($F_{7,16} = 4.81$, $P = 0.0045$) but there was no difference between categories, glyphosate-resistant ($96.8 \pm 8.5\%$) and conventional varieties ($86.2 \pm 20.2\%$) ($F_{1,16} = 0.03$, $P = 0.86$). Percentage of seedlings with root hair ranged between 67.5 and 100% among varieties (Table 2).

The percentage of seedlings with secondary roots was statistically significant among varieties ($F_{7,16} = 3.39$, $P = 0.02$). All conventional seedlings exhibited secondary roots as opposed to $94.1\% \pm 10.8$ for glyphosate-resistant seedlings, a statistically significant difference ($F_{1,16} = 6.76$, $P = 0.02$).

Varieties differed in root length ($F_{7,16} = 5.82$, $P = 0.0017$) but there was no statistically significant difference between the two categories ($F_{1,16} = 1.71$, $P = 0.21$). Average root length varied between 44.8 and 87.7 mm among varieties (Table 2) and the average for glyphosate-resistant varieties was 74.0 ± 20.4 mm relative to 48.3 ± 9.6 mm for conventional varieties.

Comparing control and glyphosate treatments

When we combined control and 80 ppm glyphosate treatment, we observed no difference in seed germination between the control ($82.1\% \pm 16.4$) and the glyphosate ($82.9\% \pm 13.0$) treatment ($F_{1,32} = 0.06$, $P = 0.80$). Seed germination did not vary significantly among varieties ($F_{7,32} = 1.91$, $P = 0.10$) and there was no statistically significant interaction between variety and treatment on their impact on seed germination ($F_{7,32} = 1.94$, $P = 0.10$). Seed germination did not differ between categories, conventional ($82.1\% \pm 15.9$) and glyphosate-resistant ($82.1\% \pm 13.7$) ($F_{1,32} = 0.25$, $P = 0.62$).

There was a statistically significant impact of variety ($F_{7,32} = 21.2$, $P < 0.0001$), glyphosate treatment ($F_{1,32} = 400.4$, $P < 0.0001$) and their interaction ($F_{7,32} = 12.54$, $P < 0.0001$) on percent root hair. The percentage of seedlings with root hair decreased from $91.5\% \pm 16.1$ (mean \pm STD) in the control to $29.8\% \pm 33.9$ for seedlings exposed to glyphosate. The interaction between variety and treatment resulted from the fact that none of the conventional seedlings exposed to a 80 ppm glyphosate solution produced any root hair while a variable percentage of glyphosate-resistant seedlings produced root hair (Tables 1 and 2).

Both variety ($F_{7,32} = 40.37$, $P < 0.0001$), treatment ($F_{1,32} = 716.84$, $P < 0.0001$) and their interaction significantly affected the percentage of seedlings with secondary roots ($F_{7,32} = 54.54$, $P < 0.0001$). While 100% of conventional seedlings exhibited secondary roots in the control, 0% did in the glyphosate treatment. All glyphosate-resistant varieties experienced a decrease in the percentage of seedlings with secondary roots in the glyphosate treatment but the reduction was less than for the conventional varieties (Tables 1 and 2).

We observed a statistically significant impact of variety ($F_{7,32} = 12.08$, $P < 0.0001$), glyphosate treatment ($F_{1,32} = 251.86$, $P < 0.0001$) and their interaction ($F_{7,32} = 3.36$, $P = 0.0084$) on root length. Root length decreased for all varieties in the glyphosate treatment but the percentage decrease was greater for some varieties (Tables 1 and 2).

Discussion

We confirmed that the conditions selected by Boyle et al. (2016), a 16h light: 8h dark regime, 80 ppm glyphosate concentration and a 14-day germination period, represented the best conditions

to grow seeds in order to separate conventional and glyphosate-resistant seedlings using a phenotypic assay. We demonstrated that the seedling phenotypic assay could be done with seeds placed in petri dishes in an incubator. Our data indicated that placing 10 seeds per petri dish provided more consistent results than 5 seeds per dish. While we observed the absence of root hair in all conventional varieties exposed to an 80 ppm glyphosate solution, in contrast to Boyle et al. (2016), we did not observe the presence of root hair in all the glyphosate-resistant seedlings exposed to this treatment. We observed variation in the percentage of seedling with root hair among varieties in both the control and the 80 ppm glyphosate treatment. We expect such variation to affect the sensitivity of the assay among varieties and, therefore, do not expect the presence/absence of root hair between glyphosate-resistant and conventional seedlings to be 100% accurate when extended to different varieties.

In the current study, the development of secondary roots was associated with glyphosate resistance and represents another useful phenotypic trait to distinguish between conventional and glyphosate-resistant seedlings. The conventional varieties examined had 100% secondary roots in the control and none in the glyphosate treatment. In the control, $94.1\% \pm 10.8$ of the glyphosate-resistant seedlings had secondary roots in contrast to $77.8\% \pm 15.1$ in the glyphosate treatment. We did not consider root width in this study because this trait tends to be strongly correlated with root length and we did not expect it would add much to the assay results.

Unlike Boyle et al. 2016, we did not observe an impact of the glyphosate treatment on seed germination. Seed germination was high in all treatments and varieties. We did not observe differences in seed germination between glyphosate-resistant and conventional varieties in the glyphosate treatment or the control.

All varieties, conventional and glyphosate-resistant, were negatively affected by glyphosate exposure in the seedling stage. The percentage of seedlings with root hair, percentage of seedlings with secondary roots and root length were all greater in the control than in the glyphosate treatments and this was true for all varieties. The only trait that did not vary among varieties and was unaffected by glyphosate exposure was seed germination. While all varieties were negatively affected by glyphosate exposure, the response of glyphosate-resistant and conventional varieties differed which permitted the development of a phenotypic assay to identify glyphosate-resistant seedlings.

Based on our germination assay experiments, we were able to determine a rule for identifying glyphosate-resistant seedlings based on phenotypic characteristics. If we score seedlings only based on the presence of secondary roots and/or root hair, we will have no false positive (i.e. scoring conventional seeds as glyphosate-resistant), but will have a false negative rate of 16.8%, meaning that up to 16.8% of glyphosate-resistant seedlings will be scored as conventional. However, if we add root length to the rule and score all seedlings with secondary roots and/or root hair that are longer than 19.0 mm as glyphosate-resistant, we will maintain a false positive rate of 0 as no conventional seedlings had roots longer than 18.6 mm in the 80 ppm glyphosate treatment and minimize the false negative error rate (i.e. scoring glyphosate-resistant seedlings as conventional). However, we still have a false negative error rate of 12.6% using this method, meaning that up to 12.6% of seedlings scored as conventional could in fact be glyphosate resistant. These false negatives represent some glyphosate-resistant seedlings with short roots and no root hairs. We therefore strongly recommend complementing the phenotypic assay with the use of AgraStrip RUR Seed & Leaf test strips. We suggest pooling seedlings scored as

conventional using the phenotypic seedling assay in groups of 20-40 and test one leaf of each; in the event of a positive result each seedling can be tested individually using the other leaf to identify those seedlings phenotypically scored as conventional that are actually glyphosate resistant. Alternatively, depending on the required level of accuracy in the experiment, one could use the error rate of 12.6% for false negative events and correct the data using this value.

As we have developed this scoring rule using four conventional and four glyphosate-resistant alfalfa varieties, we are confident that it is a robust rule, especially in terms of the absence of root hair and secondary roots on conventional seedlings grown in 80 ppm glyphosate. While our results were not as conclusive as those of Boyle et al. (2016) at distinguishing glyphosate-resistant from conventional seedlings based on the presence of root hair, we were able to confirm that the conditions used in their germination assay best facilitated separation of conventional and glyphosate-resistant seedlings based on phenotypic traits. In addition, we were able to successfully conduct all assays using simpler experimental conditions, providing a cost-effective alternative to medium-scale screenings.

This phenotypic assay can be used to measure gene flow detected by the presence of glyphosate resistance in conventional, organic alfalfa fields or fields destined for the export market or in any gene flow experiments using the glyphosate resistance gene as a marker. The assay could also facilitate the detection of the glyphosate resistance gene in feral alfalfa populations (Greene et al. 2015). The use of the phenotypic assay to detect glyphosate-resistant seeds could substantially reduce the cost of glyphosate resistance detection in large gene flow experiments. Such experiments could highlight methods to limit gene flow among GE and non-GE fields and therefore facilitate the successful coexistence of the different alfalfa seed-production markets.

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LYGUS EFFICACY TRIALS IN ALFALFA PRODUCED FOR SEED 2018.

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Insecticides were screened for their ability to control Lygus bugs and alfalfa weevil on alfalfa produced for seed and for their potential negative impact on beneficial arthropods in mid summer 2018. Field plots were established at the irrigated Agriculture Research and Extension Center near Prosser, WA. Established plots were 360 ft² (18 ft. wide and 20 ft. long) and all treatments were replicated 4 times in a randomized complete block design. Insecticides were applied to mimic grower timing at the post-bloom period of production.

Treatments were applied on August 9, 2018 using a CO₂-powered backpack sprayer equipped with a four-nozzle boom using 19.8 gallons of water per acre as a carrier. Five 180° sweeps per plot were used post-application on August 10, 14, and 24 as a means to sample Lygus and other arthropod abundance including aphids, spiders, big-eyed bugs, minute pirate bugs, assassin bugs, lace wings, lady bird beetles and weevils. Abundance counts for Lygus, spiders, damsel bugs, and alfalfa weevils are detailed in this report. The abundance of other arthropods was inconsequential.

Analysis of variance (ANOVA) was conducted on insect abundance counts for each pest on each respective sample date. The insect abundance counts for each pest by insect treatment was then compared to the untreated control population means in pair wise *t*-tests. Transform was a superior insecticide and provided control of Lygus that was consistently equal to or superior to the pyrethroids including Mustang Maxx and Warrior II. All treatment effects were done by 21 days after treatment. It should be noted that these plots were established on a 1 acre block of poorly managed alfalfa hay on the WSU IAREC Roza unit. Lygus abundance was substantial.

Section 18 Emergency Exemptions were issued for Transform (sulfoxaflor) in 2018 in Washington State and Idaho. A Crisis Section 18 was issued in Oregon. We anticipate that these 3 states will continue to submit Section 18 requests for Transform until Corteva receives registration of Transform on their Tier 2 crops. Alfalfa is among the crops on Corteva's Tier 2 list. Growers of alfalfa seed should soon have this 2nd "safer" insecticide for use on blooming alfalfa once Transform is registered via Section 3. We have determined that if growers of alfalfa produced for seed follow simple recommendations like limiting insecticide sprays to evening hours with products like Beleaf and Transform that Lygus bugs can be controlled with relative safety and minimal harm to pollinating bees. My present recommendations for Lygus control on alfalfa include a pre-bloom "cleanup spray. There are several effective pyrethroids and organophosphate products registered as well as some effective premix products that contain OPs and pyrethroids. During early bloom the insecticide of choice is Beleaf and later in bloom as the plants desiccate Transform is the insecticide of choice. Growers are limited to 2 applications of Beleaf and Transform. Some growers are seriously considering using Transform as their pre-bloom cleanup spray given Transform's efficacy and its softer footprint on beneficial arthropods. Other factors include the fact that Transform does not appear to flare mite outbreaks compared to the pyrethroids registered for use on alfalfa grown for seed. Most alfalfa seed fields require mid-summer acaricide sprays after pyrethroids have been used in late spring.

	Product	Rate/acre	
1	Control		Fl oz/acre
2	Beleaf 50 SG	2.8	oz/acre
3	Sivanto*	14	Fl oz/acre
4	Naled	16	Fl oz/acre
5	Transform	1.5	oz/acre
6	Transform	2.25	oz/acre
7	Stallion	11.75	Fl oz/acre
8	Cobalt Advanced	37	Fl oz/acre
9	Warrior II	1.92	Fl oz/acre
10	Mustang Maxx	4	Fl oz/acre
11	Biological Soil & foliar	3	Quarts

Date	Days after treatment	Treatment	Lygus Adult	Lygus large nymph	Lygus small nymph	Spider	Damsel bugs	Alfalfa weevil
8/10/2018	1	Control	19	11.5	18.25	2	1	1.25
8/10/2018	1	Beleaf 50 SG	9.5	8.25	6.25*	5	2.25	1.75
8/10/2018	1	Biological Soil & foliar	19.75	11.5	15.25	3.5	1.75	0.5
8/10/2018	1	Cobalt Advanced	5.5*	4.75*	0.75*	3.25	0	1.25
8/10/2018	1	Mustang Maxx	4.25*	5.5	9.75	1	0	2.75
8/10/2018	1	Naled	19.5	9.25	7	3.5	0	0
8/10/2018	1	Sivanto	29.25	8	18.75	3.25	3	0.5
8/10/2018	1	Stallion	3*	1.75*	3.25*	2	0.25	0.25
8/10/2018	1	Transform1.5	8.25*	4*	5.25*	5.75	3.25	0.5
8/10/2018	1	Transform2.25	6.25*	2.25*	4.25*	6.5	1.75	0.25
8/10/2018	1	Warrior II	4.75*	6.5	10.5	1.75	0	2
8/14/2018	5	Control	12.25	8.5	8.25	3.5	0.75	1.25
8/14/2018	5	Beleaf 50 SG	14.5	5	6.25	6.5	1	1.75
8/14/2018	5	Biological Soil & foliar	12	5.25	3.75	6.75	3.75	0.75
8/14/2018	5	Cobalt Advanced	6.75*	2*	1*	0.25	0.25	0
8/14/2018	5	Mustang Maxx	4.75*	7.5	7	1.25	0.5	6.5
8/14/2018	5	Naled	21.25	7.75	5.5	7.25	2.5	0.5
8/14/2018	5	Sivanto*	15.75	6.5	7.5	4.5	1.25	1.75
8/14/2018	5	Stallion	7.75	2.25	1.5*	5.25	0	4
8/14/2018	5	Transform1.5	12.75	0.5*	0.5*	7	0.25	1
8/14/2018	5	Transform2.25	8	2.5*	2.25*	5	1	1.5
8/14/2018	5	Warrior II	8.25	8.5	3.5	3.75	1	9
8/24/2018	15	Control	17.75	12.5	10	3.25	1.25	2
8/24/2018	15	Beleaf 50 SG	10.75	5.25	15	4.75	0.75	1.25
8/24/2018	15	Biological Soil & foliar	13.5	6.5	14.5	4.5	1.75	0.5
8/24/2018	15	Cobalt Advanced	11.25	1.75*	11	2	0.25	1
8/24/2018	15	Mustang Maxx	11.25	8.25	23.75	2	0	3
8/24/2018	15	Naled	12.5	3.75	16	4.75	1.75	0.75
8/24/2018	15	Sivanto*	13.25	10.25	19.75	2.5	0	0.75
8/24/2018	15	Stallion	13.5	0.75*	24	2.25	0.5	3.25
8/24/2018	15	Transform1.5	21	1.25*	21.25	3.75	1	1
8/24/2018	15	Transform2.25	16.25	4.5	14.75	4.75	0.25	1.25
8/24/2018	15	Warrior II	15.5	4.75	10.25	1.25	0.75	4

*/ treatment means are significantly lower than in the untreated Control plots

Status of the Western Alfalfa Hay Market

Seth Hoyt

2018 in Review

Last year at the Western Alfalfa Symposium in Reno I said that export buyers would be bigger force in the western alfalfa hay market in 2018. At the start of the season export buyers were active in the southern California desert in March and April with buyers for the Saudi Arabian market the most aggressive. Export buyers were aggressive in other areas of the west when new crop alfalfa hay trading began in May. While there was dairy demand, milk prices in the \$13 to low \$14 range in California kept a lid on early dairy demand as dairies were struggling financially. Demand picked up from some dairies in May as the milk price projection was \$16.00 per hundredweight and higher by late summer. Unfortunately, by June and July the outlook for stronger milk prices faded and so did dairy demand. But with strong export demand the alfalfa hay market was cruising along without much problem until July when China instituted tariffs on imports of alfalfa hay. This was a big deal because in 2017 around 45 percent of alfalfa hay exports from the west coast went to China. So, after losing dairy demand because of low milk prices, now export demand tapered off. In past years there were only a few times when dairy and export demand for alfalfa hay in the west fell at the same time, but they did in 2018. Consequently, prices on Premium to Supreme export and dairy hay declined. Premium export alfalfa hay in Washington dropped \$15 per ton from the high of \$194 average in the month of May to \$178.67 per ton average in October. Supreme dairy alfalfa hay delivered to Tulare-Hanford California dairies dropped \$20 per ton from May to September. Alfalfa hay in some areas of the west such as Nevada and Utah that would have been normally purchased by export and dairy buyers was sitting on the farm with very little if any demand. While trading picked up a little in October, particularly in Washington and Idaho for export and dairy alfalfa hay it was still not the normal demand you would see for better quality late season alfalfa hay. Some of this hay was testing very strong, higher than normal.

Feeder/Dry Cow Versus Premium/Supreme Alfalfa Hay

One of the interesting stories in the 2018 alfalfa hay season in the west was the demand and prices on Fair quality feeder/dry cow alfalfa hay. Due to better than normal hay production weather through the season there was not the normal production of Fair quality alfalfa hay. This was significant because not only is this quality of hay used for dairy dry cows but also by feedlots and beef cows in the west. Additionally, export demand for this type of hay improved later in the season for some markets, including the UAE, and the price per ton did not drop like on better quality alfalfa hay in some

areas. For example, Fair export alfalfa hay in Washington in late October at \$160 to \$165 per ton was \$25 to \$30 per ton higher from both early July of this year and at the same time a year ago on limited supplies. There was concern by some in the hay industry that if winter conditions turned severe in Idaho & Washington and surrounding States this winter that there would not be enough feeder hay to meet the demand from beef cattle producers. Fair quality alfalfa hay for dry cows delivered to Tulare-Hanford dairies or feedlots in late October brought \$205 to \$220, \$20 to \$30 higher than the same time last year. Conversely, Supreme alfalfa hay delivered to Tulare-Hanford California dairies in late October brought \$265 to \$280 delivered, \$10 to \$20 per ton lower than a year ago. Fair quality feeder hay in Idaho in late October brought \$130 to \$140 fob stack, \$30 to \$35 higher than a year ago and compared to \$110 to \$125 in June and July of this year. Supreme alfalfa hay prices in Idaho in late October, while up \$10 to \$15 from a year ago were down \$15 to \$25 from early in the season.

Hay Carryover

It is a major challenge to predict how much hay in the west will be carried into 2019 versus a year ago. If you looked only at hay production in the seven western states you would say hay stocks would be down going into 2019 because production was down in most States. But because of low milk prices and tariffs on alfalfa hay going to China, hay trading and movement were altered. In Idaho, where alfalfa hay production was higher than 2017 there were short supplies of feeder hay but there was some better-quality alfalfa hay that had still not sold in mid fall. Also, in Eastern Idaho there was export alfalfa hay that sold early in the season that had still not moved. While hay stocks could be down in some areas of the west, in other areas they may not because of hay that has not sold or shipped. There was better quality alfalfa hay in Nevada and Utah that has still not traded in the last week of December. As mentioned above, on a normal year much of this hay would be sitting on dairies in central California or have been purchased by exporters and some already moved overseas. We know that feeder hay supplies are short, particularly in Idaho, Washington, and Utah. In California, there are some areas where there seems to be more hay in farmer hands than a year ago, but it is hard to call a bigger carryover when this hay would normally be sitting on dairies if the dairies had money to buy hay. That is the challenge trying to come up with hay carryover in central California. Also, because of lower alfalfa hay production in California in 2018 these supplies of hay on farms may not translate into a burdensome carryover. It is one of those years where USDA's December 1 Hay Stocks report for the west which will be released the second week of January 2019 will be the best source of hay supplies that will be carried into next year.

Outlook for 2019

It is years like this that made me stop trying to predict where hay prices will go in the future. If alfalfa hay prices, except retail, are going to hold where they are or get better I believe two things have to happen: milk prices must increase to levels where dairies are making a profit and tariffs are lifted on alfalfa hay shipments to China. It hasn't happened very often but when dairy and export demand both turn very light at the same time as they did at mid-season it can really change the demand for alfalfa hay. We saw this in Nevada and Utah and central and northern California after mid-summer. The one encouraging development is the demand for alfalfa hay for the Middle-East which sparked demand and stronger alfalfa hay prices in Washington later in the fall.

The dairy situation has not been good and unfortunately as I write this in the second week of November, it looks even worse in the next four to five months. Many California dairies are under water financially and further reductions in milk prices will push more dairies out of business. This is why I see no incentive for alfalfa hay growers in the central and northern valley of California to plant more alfalfa hay in 2019. What is the incentive when your main buyer of alfalfa hay is in such financial stress? While there can be export demand, the fact is dairies are the main buyer of alfalfa hay in central and northern California. As long as almonds are making money look for more almond trees to be planted in the coming year and less alfalfa hay in the central and northern valley of California. In the Imperial Valley, indications point to higher alfalfa hay acres in 2019 with acres on November 5, 2018 up 2 percent from a year ago.

In other areas of the west like Washington, Idaho, and Utah alfalfa hay was one of the more profitable crops and early indications point to mostly unchanged alfalfa hay acres in 2019 but this is very preliminary. Export demand will again depend on how long it will take to settle the trade war with China. We have seen export demand for China becoming a bigger factor in Idaho with another new press being built in Eastern Idaho mainly for this market. There is hope that the trade war with China will be settled by January 1, 2019. While the China market is also a big factor in the Washington alfalfa hay market, the demand for alfalfa hay for the Middle-East has picked up and sources think this could grow in the coming year. In the southern desert of California and Arizona as well as other areas in the west there will be more demand for alfalfa hay for Saudi Arabia. Sources have said the Saudi Arabian government will require that irrigation of larger alfalfa farms be discontinued after the middle of December 2018. This will increase demand for alfalfa hay in the western U.S. in 2019. We have seen increased purchases of alfalfa hay destined for the UAE in recent months, but it is questionable how long this demand will continue after the Trade war with China is settled. China has been outbidding the UAE for alfalfa hay in some parts of the world.