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WESTERN ALFALFA SEED GROWERS
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PROGRAM

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Sunday, January 29

5:00 PM – 6:30 pm – Opening Social – *Bel Air II*
Sponsored by – JWM Leafcutters

Monday, January 30

8:00 AM – **Registration Desk Opens** – Trade Show – *Bel Air Foyer*

Session 1 – Pesticide Credit Session – *Bel Air I* – Moderated by Shane Johnson

8:30 AM – **Conference Kickoff –** Shane Johnson, Western Alfalfa Seed Growers Association
8:40 AM – **Herbicide Resistance: Implications for Weed Management** – Dr. Drew Lyon, Professor and Endowed Chair Small Grains Extension and Research, Weed Science, WSU
9:20 AM – **Spray Nozzle Technology & Droplet Size Management** – Mike Powers, Northwest Regional Sales Manager, TeeJet Technologies
10:10 AM – **Break/ Spray Table Presentation – Raffle Drawing** – *Coffee and Breaks – Bel Air Foyer*
Sponsored by – DSV/Northstar Ltd.

Session 2 – *Bel Air I*

10:30 AM – **NAFA Update** – Beth Nelson, President, National Alfalfa and Forage Alliance
11:10 AM – **Grower and Industry Survey –** Shane Johnson, WASGA Executive Director
11:50 AM – **Lunch & Raffle Drawing – *Bel Air II***
Sponsored by – Seed Services

Session 3 – APRI Research Session *Bel Air I* – Moderated by Theresa Pitts-Singer

1:20 PM – **Informing effective alkali bee (Nomia melanderi) management decisions with novel microsatellite markers and population genetic analysis**– Jon Koch, USDA Logan, UT
1:40 PM – **Comparing pesticide exposures of two important alfalfa pollinators, alkali bees and alfalfa leafcutting bees** – Kelsey Graham, Research Entomologist, USDA-ARS PIRU, Logan, UT
2:00 PM – **Assessment of Seasonal and Yearly Variability of Alfalfa Leafcutting Bee (Megachile rotundata) Parasite Infestation in Seed Production Fields**– Lindsie McCabe, USDA Logan, UT
2:20 PM – **Insight into causes of death for Alkali bees AND Research update from PIRU** – Diana Cox-Foster, USDA Logan, UT
2:40 AM – **Break – Raffle Drawing** – *Coffee and Breaks Sponsored by – DSV/Northstar Ltd.*
3:00 PM – **Enhancing & Protecting Populations of Alfalfa Seed Pollinators** – Doug Walsh, WSU, Prosser, WA
3:20 PM – **Alfalfa Weevil and Lygus Bug Control** – Doug Walsh, WSU, Prosser, WA
3:40 PM – **Maternal Effects and Management of Alfalfa Leafcutting Bees** – Makenna Johnson Bird, Biological Science Technician at USDA Agricultural Research Service Pollinating Insect Research Unit
4:00 PM – **Raffle Drawing/Announcements**
4:00 PM – 5:30 PM – **Presenter's Meet and Greet Social & Researchers Poster Panel – *Bel Air II***
Sponsored by – Mr. Pollination Services

Tuesday, January 31

8:00 AM – **Registration Desk Opens** – Trade Show Ongoing – *Bel Air Foyer*

Session 4 - *Bel Air I*

8:30 AM – **Welcome** – Shane Johnson, Western Alfalfa Seed Growers Association
8:40 AM – **Pollinator Partnership – Bee Friendly Farming Certification Program** – Cody Wilson, Bee Friendly Farming Pacific Northwest Associate, Pollinator Partnership
9:00 AM – **"Tripping" out with alfalfa leafcutting bees: A career's perspective** – Theresa Pitts-Singer, USDA, Logan, UT
9:40 AM – **Canadian Seed Report –** Kurt Shmon, President/CEO, Imperial Seed Ltd, Winnipeg, MB Canada
10:20 AM – **Break – Raffle Drawing** – *Coffee and Breaks – Bel Air Foyer*
Sponsored by – DSV/Northstar Ltd.
10:40 AM – **Challenges & Opportunities for Alfalfa Moving Forward -** Dennis Hancock, USDA-ARS, U.S. Dairy Forage Research Center - Madison, WI USA
11:20 AM – **Keynote – Preparing your Farm for Future Markets** - Jon Paul Driver, Economic Specialist, Washington State University
12:20 PM – **Raffle Drawing/Announcements/Conference Adjournment**

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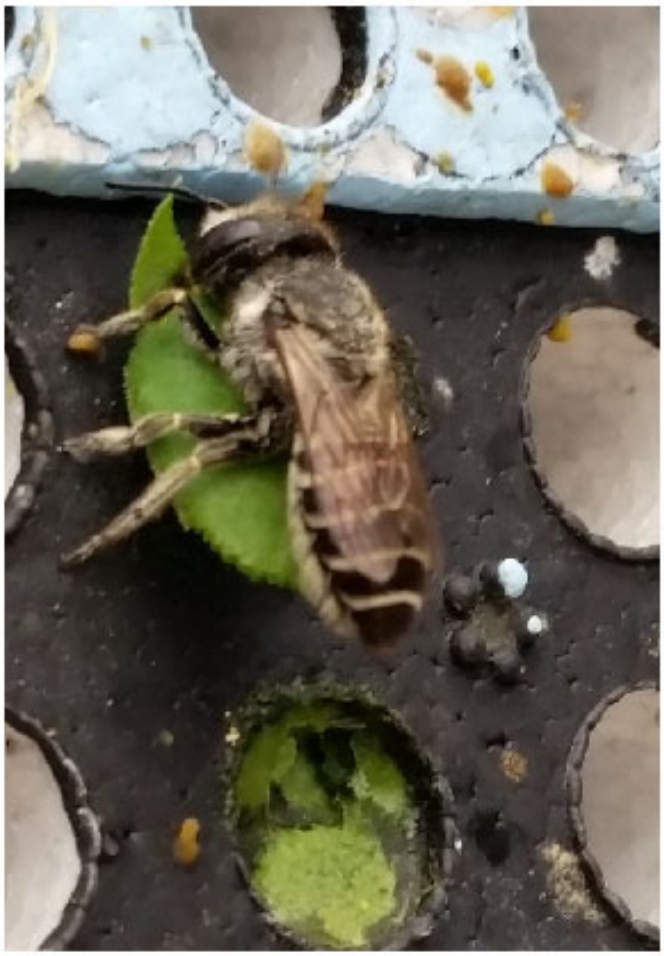

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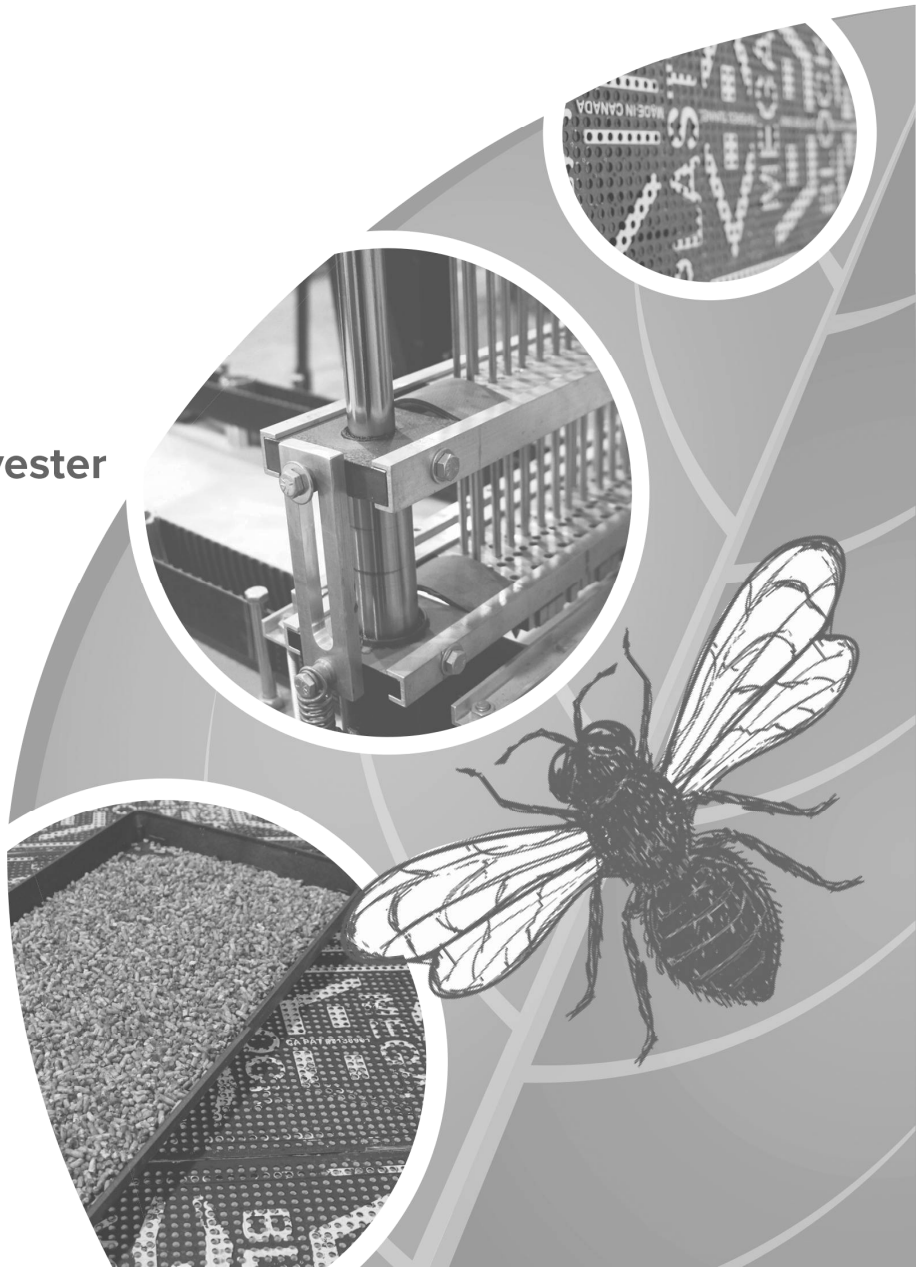

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Pictured: RMA Administrator Marcia Bunger with faculty of the University of Arkansas at Pine Bluff

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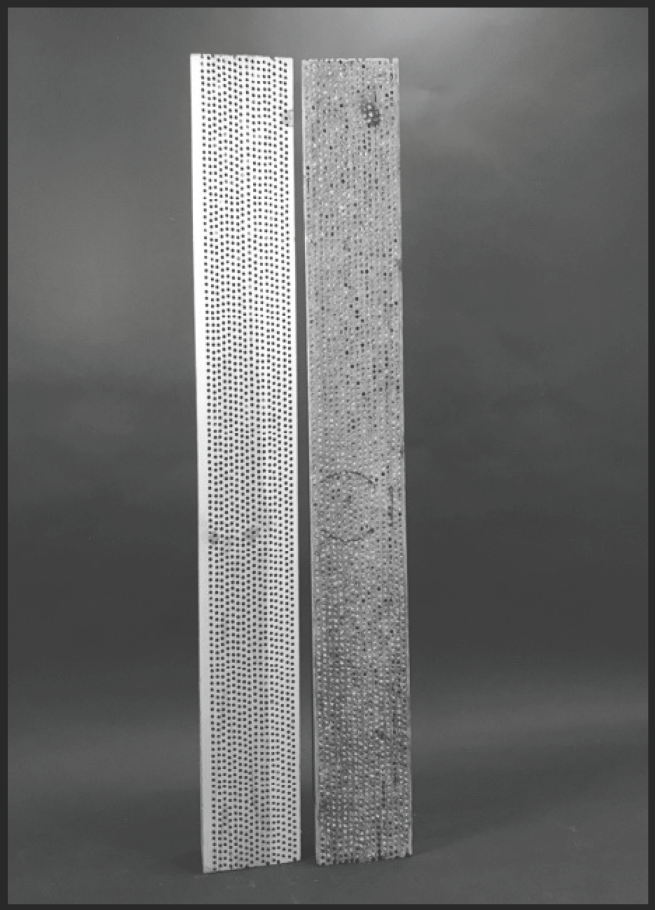


BEST MANAGEMENT PRACTICES FOR

MANAGING HERBICIDE RESISTANCE

PNW754

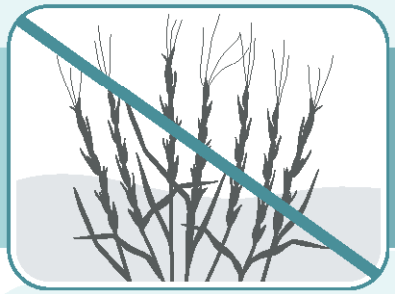
Herbicide resistance is a problem that has quickly spread throughout the wheat growing regions of the inland Pacific Northwest. Overreliance on herbicides for the management of weeds is a major cause of herbicide resistance. Integrated weed management relies on a wide range of practices to manage weeds and slow the development and spread of herbicide resistance. This publication presents growers and farm managers with best management practices (BMPs) that can be incorporated into farming systems to manage herbicide resistance. A table toward the back of the publication (Table 3) allows readers to identify practices that are already being used and additional practices that should be implemented.

Drew J. Lyon, Professor and Endowed Chair Small Grains Extension and Research, Weed Science, Department of Crop and Soil Sciences, Washington State University
Judit Barroso, Assistant Professor, Department of Crop and Soil Science, Oregon State University
Joan M. Campbell, Principal Researcher, Department of Plant Sciences, University of Idaho
Douglas Finkelnburg, Extension Educator, Nez Perce County Extension, University of Idaho
I.C. Burke, Professor and R. James Cook Endowed Chair, Department of Crop and Soil Sciences, Washington State University

START CLEAN

Use agronomic practices that limit the introduction and spread of weeds. In other words:

Prevent weed problems before they start.

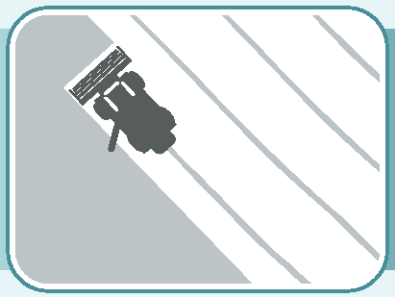


Seed into weed-free fields and keep them weed-free.

One of the fundamental ways to prevent development of herbicide-resistant weeds is by controlling all weeds prior to seeding a crop and by keeping fields clean (weed-free) throughout the growing season. Because herbicide resistance is thought to be present at very low frequencies (for example, one plant in a million) before selection pressure from herbicides are applied, keeping weed numbers low reduces the chance of that rare resistant plant being present in your field.

Remove or cut weeds before seed set.

Reduce the soil seedbank (the reserve of viable seeds present on the soil surface and scattered throughout the soil profile) by removing or destroying any weed seed that is produced. Preventing weeds from setting seeds may not benefit the current crop but will reduce the weed seedbank over time. The old saying "One year's seeding, seven years' weeding" may be even more important when talking about herbicide-resistant weeds because tools to manage those biotypes might be very limited.



Manage weed seed at harvest and after harvest to prevent weed seedbank buildup.

Practices to control weed seed at harvest have proved to be useful to reduce the soil seedbank, particularly of potential herbicide-resistant weeds that survive in-crop herbicide applications. Examples of some of these practices are the bale direct systems, chaff lining and chaff tramlining, or impact mills integrated into combines to destroy the weed seeds in the chaff (see

Harvest Weed Seed Control: Applications for PNW Wheat Production Systems [Lyon et al. 2019]). Harvest weed seed control practices target the control of species that retain a significant amount of seeds at crop maturity such as tumble mustard or common lambsquarters. However, for species that set seed after harvest, like Russian thistle or prickly lettuce, it is very important to control these weeds after harvest to prevent larger infestations in those fields and neighboring fields into which they may move or disperse seed.

Applying composted livestock manure to fields can be a good way to improve soil organic matter and provide beneficial nutrients. However, manure can also be a source of weed infestations. Manure should be correctly composted to ensure sufficient heating to kill weed seeds. Turn and mix the compost pile to ensure all the potential weed seeds are exposed to high temperatures (130–145 degrees Fahrenheit).

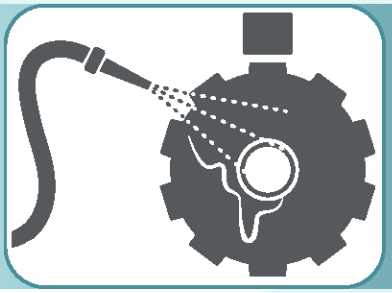
Compost livestock manure.

Use weed-free seed.

Seeding crop seed contaminated with weed seeds has been the most common method of spreading weeds for centuries. To minimize this risk, use high-quality, weed-free seed, or purchase certified seed.

Understand weed biology, particularly timing of seed germination, seed dormancy, and seed longevity.

Although practices to either break down seed dormancy or encourage seed germination to reduce the soil seedbank are not going to be a "silver bullet," they will help to both decrease the probability of having herbicide resistance problems and reduce overall weed pressure. An understanding of the conditions that favor or limit germination (to manage seed longevity and seed dormancy) in problematic weed species will help to optimize herbicide application timings and agronomic practices (seeding dates, residue management, tillage practices, crop rotations) that will lead to improved control.



Prevent field-to-field and within-field movement by starting equipment usage in weed-free areas and by cleaning equipment after use.

Prevention is the most effective method of dealing with weeds. Restrict the opportunity for new weeds to invade and spread. Do not allow machinery or vehicles to enter your property unless they are clean. Restrict the movement of vehicles and machinery on your property during periods when seeds are likely to spread. If a weed infestation already exists in a field, start working from the clean areas, and leave the infested areas for last. Clean the equipment after operating it in infested areas, particularly harvesting equipment.

Control weeds in borders to prevent weed influx into the field.

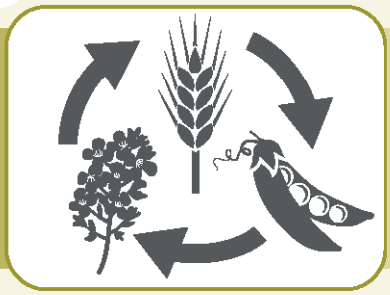
Weeds in unmanaged areas, including field margins, roadsides, rights-of-way, and ditch banks, can serve as a source for the introduction and movement of new weed species including herbicide-resistant weeds. Allowing weed seed production in field borders can have long-term effects on the seedbank, especially when outcrossing occurs with resistant populations near a field, allowing the spread of resistance through pollen and seed movement.

STAY CLEAN

Help the crop compete with weeds. In other words:

Help the crop "choke out" weeds.

Use crop competitiveness and other cultural management strategies, including crop rotation.



Seed-to-soil contact is key to maximizing germination potential. Ensure row cleaners, packing wheels, and other seeder attachments are correctly adjusted to gently firm the soil without overly displacing it or excessively packing it. Seeding into adequate, not excessive, soil moisture promotes quicker germination and lowers some disease pressures. Manage crop residue to avoid "hair-pinning" straw while seeding.

Prepare a firm, moist seedbed.

Delay seeding until soil temperatures and moisture are optimal for crop emergence. Avoid cool and excessively wet seeding conditions to maximize even stand development and minimize opportunities for weeds to emerge (see Table 1).

Seed at an optimum time for rapid germination and emergence.

Choose varieties that emerge quickly, grow rapidly, and swiftly form a crop canopy. In addition, varieties with robust disease tolerances to known site problems will minimize risks of uneven stand development.

Select competitive crop cultivars.

SOIL TEMPERATURES FOR RAPID CROP GERMINATION

	Minimum (°F)	Optimal (°F)
Wheat	37	54–77[a]
Spring Barley	40	55–75[b]
Dry Field Pea	40	60–70[c]
Chickpea	41	50–59[d]
Sp. Canola	41	50[e]

Sources: [a]Evans et al. (1975); [b]Stark (2003); [c]Oelke et al. (1991); [d]Corp et al. (2004); [e]Oplinger et al. (1989).

Table 1. Soil temperatures for rapid crop germination.

Use high-quality seed.

Choose larger seed with a sufficient test weight and known germination potential from a trusted source, such as certified seed. Avoid seed with excessive small, shriveled kernels, even if it has a good test weight. Plump seeds emerge quickly and will be more resilient in adverse seeding conditions.

Use treated seed.

Seed treated with appropriate fungicides and insecticides is protected from pests that can slow early growth and cause stand loss that results in reduced competition with weeds.

Use higher seeding rates.

Higher seeding rates cause plants to be more competitive for resources providing better weed suppression through competition (Figure 1).

Use narrowest feasible row spacing.

Narrower row spacing allows crops to form a canopy more quickly and suppress weeds earlier in the season.

Seed on the shallow side of the recommended seeding depth.

Shallower seeding results in faster seedling emergence and leads to earlier establishment of the crop canopy to suppress weed emergence.

Apply fertilizer to promote crop growth and competitiveness.

Do a soil test to determine optimal fertility for your yield goals. Apply fertilizer in bands below the seed when possible to maximize fertility for the crop and minimize resource competition from weeds. Use a starter fertilizer to maximize seedling emergence speed and early season vigor.

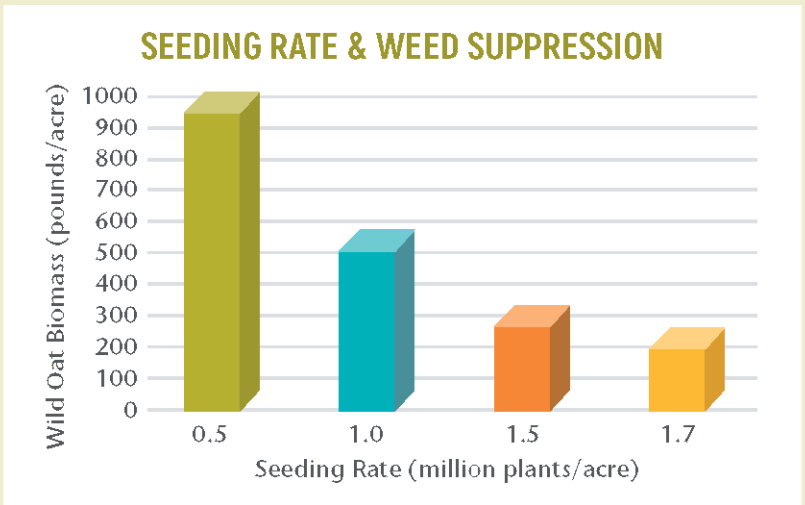


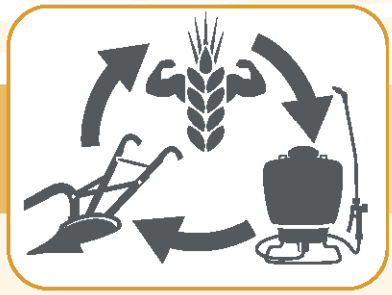
Figure 1. Wild oat biomass in spring barley at different seeding rates in late June.

Adapted from *Crop Density—A Weed Management Tool* (Veseth 1988).

REMAIN CLEAN

Use practices that keep weeds "off balance." In other words:

Do not allow weeds to adapt.



Diversify weed management practices.

Winter annual weeds that germinate during late fall can be controlled before seeding spring crops. Fall-seeded crops compete well with annual weeds that germinate during late spring or summer. In general, more herbicides are available to control grass weeds in broadleaf crops or to control broadleaf weeds in grass crops. Crops that grow taller rather than shorter, crops that are seeded in the winter rather than spring, and crops that tiller higher and faster (e.g., barley versus wheat) often compete against weeds better. Incorporation of a perennial crop, especially a forage, is competitive against annual weeds. Increase the number and diversity of crops and the length of the rotation.

Rotate crops with varied life cycles and seeding dates.

Rotating or combining MOA helps only when all herbicides in the rotation or tank mix have activity on the target weed species. Following the initial herbicide treatments, subsequent herbicides can be used to control resistant plants that have emerged.

Rotate or combine herbicides with different mechanisms of action (MOA) and activity on *each weed species.*

The use of herbicide-resistant crops allows for increased herbicide rotation and often control of a weed species that has no other herbicide management options. Avoid overuse of one type of herbicide-resistant crop. For example, do not use Clearfield or CoAXium wheat more than once in three years, and always use them in systems that include preemergence herbicides.

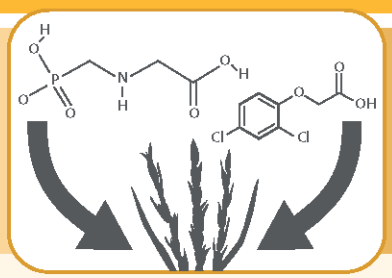
Rotate use of herbicide-resistant crops.

Annual grasses and perennial weeds tend to predominate in no-till systems, whereas annual broadleaf weeds tend to flourish in tilled systems. Occasionally changing tillage practices can prevent one or more weed species from proliferating in a field. A light harrow or cultivation of the soil can induce weed seed germination and allow subsequent control with herbicides. Herbicide rotation can be expanded by using herbicides that require mechanical incorporation.

Rotate tillage practices.

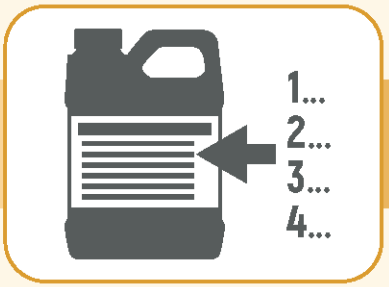
Cover crops delay or prevent weed seed germination and compete with weeds that do grow.

Incorporate cover crops.



Use multiple herbicide mechanisms of action that are effective on troublesome or herbicide-resistant weeds.

Research has shown that applying two herbicides that provide good control of the same weed species will reduce the likelihood of herbicide resistance. They must have a different mechanism of action and a different metabolic breakdown process in the plant. They should be applied in a way that minimizes antagonism. This could include a preemergence herbicide plus a postemergence herbicide *if* both herbicides will still be active at the same time. Weed seed production will likely be lower when herbicides are combined rather than just rotated.



Follow the herbicide label.

Use the correct rate at recommended weed sizes. Reduced herbicide rates, or application to larger than recommended weeds, might increase the incidence of resistance. Optimize spray application timing, carrier volume, and adjuvant use for better control.

Scout fields routinely.

Closely monitor which weeds are present and the outcome of herbicide treatments. This will tell you which weed species are in the field, where they are, and the severity of the infestation. Proper weed identification is key. Scout early and scout often! Most weeds are easier to control when small. Herbicides rarely work against mature weeds. Scout before seeding to ensure crops are planted into a weed-free field and soon after seeding to assess the efficacy of treatments and decide on further treatments. Take note if the area of a single weed species is spreading, if an herbicide application failed on a single weed, and if some weeds of the same species remained alive while others died. Contact your local university or Extension educator if resistant weeds are suspected or if you need help with weed identification.

Know and understand the effects of weed management inputs on *each weed species*!

RESOURCES

Table 2 summarizes how the choice of management options influences the chance for developing herbicide resistance in a weed species. Use it to help you assess how likely your management practices are to result in the selection of herbicide-resistant weed biotypes on your farm.

Table 2. Risk of resistance on a per species basis.

	Risk of Resistance		
Management Option	**Low**	**Moderate**	**High**
Herbicide mix or rotation in cropping system	> 2 mechanisms of action	2 mechanisms of action	1 mechanism of action
Weed control in cropping system	Cultural, mechanical, and chemical	Cultural and chemical	Chemical alone
Use of same mechanism of action per season	Once	More than once	Many times
Cropping system	Full rotation	Limited rotation	No rotation
Resistance status to mechanism of action	Unknown	Limited	Common
Weed infestation	Low	Moderate	High
Control in last three years	Good	Declining	Poor

Adapted from *A Herbicide Resistance Risk Matrix* (Moss et al. 2019).

Use Table 3 to indicate those integrated weed management practices you are already using and those that you intend to implement in the current or coming season. Try to identify at least one new management practice listed under each of the three integrated weed management principles that you will try to implement.

Table 3. Best management practices (BMPs) for managing herbicide resistance on your farm.

Practice	Already doing	Will implement
Use agronomic practices that limit the introduction and spread of weeds		
Remove or cut weeds before seed set		
Manage weed seed at harvest and after harvest		
Compost livestock manure		
Use weed-free seed		
Understand weed biology		
Prevent field-to-field and within field movement		
Control weeds in borders		
Help the crop compete with weeds		
Prepare a firm, moist seedbed		
Seed at optimum time		
Select competitive crop cultivars		
Use high quality seed		
Use treated seed		
Use higher seeding rates		
Plant on the shallow side of the recommended seeding depth		
Apply fertilizer to promote crop growth and competitiveness		
Use practices that keep weeds "off balance"		
Rotate crops with varied life cycles and seeding dates		
Rotate or combine herbicides with different mechanisms of action		
Rotate use of herbicide-resistant crops		
Rotate tillage practices		
Incorporate cover crops		
Use multiple herbicide mechanisms of action		
Follow the herbicide label		
Scout fields routinely		
Know and understand the effects of weed management inputs		

University of Idaho | Oregon State University | Washington State University

MANAGING HERBICIDE-RESISTANT WEEDS IN THE PACIFIC NORTHWEST

Best Management Practices (BMPs) to manage herbicide-resistant weeds are critical to the long-term sustainability of wheat production in the Pacific Northwest. Using BMPs are the most effective way to manage weeds, including herbicide-resistant weeds, especially when incorporated into a long-term weed management plan.

Start clean!

- Plant into weed-free fields and keep them weed-free.
- Plant weed-free crop seed.
- Understand weed biology, particularly timing of seed germination, seed dormancy, and seed longevity.
- Prevent field-to-field and within-field movement by starting equipment usage in weed-free areas and by cleaning equipment after use.
- Control weeds in borders to prevent weed influx into the field.

Stay clean!

- Scout fields routinely, and closely monitor the outcome of herbicide treatments. The sooner problems are detected, the better the chance you can adjust your management strategy.
- **Use multiple herbicide mechanisms of action (MOAs) that are effective on troublesome or herbicide-resistant weeds.**
- Follow the herbicide label – use the correct rate at recommended weed sizes.
- Diversify weed management practices – prevent weed seed production and reduce weed seeds in the soil seed bank.
- Use crop competitiveness and other cultural management strategies, including crop rotation.
- Use mechanical management practices, as needed.
- Manage weed seed during and after harvest to prevent weed-seed bank buildup.
- Know and understand the effects of the weed management inputs on *each weed species*.

HERBICIDE RESISTANT WEEDS IN THE PACIFIC NORTHWEST

** Although there are no known herbicide resistant Canada thistle in the PNW, it is a critical export contaminant.*

If weeds are present after application, determine the reason! *Consider the following:*

- **Field History – has the treatment worked before?**
- **Weed Biology – were weeds present at application?**
- **Environment – weather conditions for herbicide activity?**
- **Application Problems – are there clear patterns?**
- **Crop Cultural Practices – is the crop vigorous?**
- **Herbicide Resistance – seek support for suspected herbicide-resistant populations!**

Seek support!

Contact your local cooperative extension office for help creating a weed management plan or if your current plan is ineffective, or see EM108: Advances in Dryland Farming in the Inland Pacific Northwest, Chapter 9, for an approach to creating such a plan.

EM108

Stay informed! Visit the resource pages of the respective universities:
UIdaho: extension.uidaho.edu/crops.aspx; OSU: agsci.oregonstate.edu/cbarc/weeds; WSU: smallgrains.wsu.edu
to stay current with developments in herbicide resistance and resistance management in the region.
For specific questions or concerns, contact the WSU Weed Science Team (509.335.1719; smallgrains@wsu.edu).

The authors acknowledge that many of the best management practices discussed in this publication are from the journal article titled *Reducing the Risks of Herbicide Resistance: Best Management Practices and Recommendations* (Norsworthy et al. 2012).

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Project Title: Informing effective alkali bee (*Nomia melanderi*) management decisions with novel microsatellite markers and population genetic analysis

Year(s) of Study: 1 year, March 2022 - February 2023.

Lead Investigator / Affiliation:

Jonathan Koch, Research Entomologist, USDA-ARS Pollinating Insects – Biology, Management, & Systematics Research Unit, Logan, UT.

Collaborating Investigator(s) / Affiliation(s):

Kelsey Graham, Research Entomologist, USDA-ARS Pollinating Insects – Biology, Management, & Systematics Research Unit, Logan, UT.

Norah Saarman, Assistant Professor, Utah State University, Department of Biology, Logan, UT.

Introduction.

Alfalfa has the third greatest production value of any crop in the United States, and over 15 million acres were harvested in 2021 (USDA National Agriculture Statistics Service, 2021). Production of alfalfa seed is dependent on bee pollination to produce marketable yields. In WA, growers in the Touchet-Gardena-Lowden (TGL) area have been managing alkali bees (*Nomia melanderi*), an effective pollinator of alfalfa, for over 50 years in bee beds adjacent to the seed fields (Cane, 2008). However, local populations of alkali bees have diminished in recent years (D. Walsh, pers. comm.), reducing their availability to growers. Possible causes of decline include exposure to pesticides, reductions in available forage (alfalfa acreage), build-up of pathogens, and genetic bottlenecks which can cause inbreeding. Population declines of managed alkali bees represent a significant loss in investment for growers who construct and maintain these beds, and a loss in future pollination potential.

To enhance management decisions of the TGL area alkali bee population, we identified 35 novel microsatellite markers with the alkali bee genome. Of these markers, we successfully demonstrated the capacity of these markers to amplify in alkali bees that were collected in the TGL in 2022. These genetic markers will be useful in supporting downstream population genetic analyses that estimate underlying genetic diversity, inbreeding, and migration rates. Ultimately, our continued research with these novel microsatellite markers will support the goal of ensuring a sustainable alkali bee population for pollination services to alfalfa in the TGL and beyond.

Research Objectives.

In this report, we present the status of our proposed research objectives:

Objective 1. Identify microsatellite markers using the published alkali bee genome.
Objective 2. Determine population structure (degree of genetic isolation), and rates of inbreeding and population bottlenecks (loss of genetic diversity).
Objective 3. Estimate migration rates among beds.

We were able to complete Objective 1 of our proposed research, and we are confident that we will be able to use what we gained in Objective 1 to complete the goals of Objectives 2 and 3. Thus, in our report, we do not provide any research updates for Objectives 2 and 3.

Methods

Field work. To achieve the goals of our research, we worked with TGL growers to collect $n = 309$ tissue samples from alkali bees from nine bee beds in July 2022 (Figure 1). These samples were used to provide DNA for microsatellite development and downstream genetic analysis. Specimens were netted and subsequently immobilized on ice to induce a chill coma. The right mid-leg was then excised from the mesosoma using a pair of ethanol sterilized forceps. Midleg tissues were placed in 70% EtOH in the field on ice and in a dark cooler. The individual was then allowed to recover from the chill coma and escape. Upon return to the lab, the midleg tissues were placed in the -20 C. The description and location of the bee beds are identified in Table 1.

Table 1. Alkali bee bed locations sampled for genetic analysis in TGL. M = Male, F = Female.

Bee Bed	# of bees	Sex
Riverside	36	M & F
Russel	45	M & F
Heusby	27	F
Byerley	30	F
Henry Garbe	33	F
Buckley	35	F
Watson	33	F
Anderson	35	F
Inman	35	F

In addition to the tissue obtained from the alkali bee beds, we also obtained material from a non-managed ("wild") bee in Challis Hot Springs (Challis County, Idaho) on 22 July 2022. In total we obtained $n = 29$ specimens. These specimens were collected by Dr. James Cane (Research Entomologist emeritus, USDA-ARS Pollinating Insect Research Unit).

Figure 1. Olivia Steinmetz (USDA-ARS field technician) netting alkali bees at a managed bed.

Museum work. In addition to tissue samples from field collected specimens, we sampled tissue from historic alkali bees housed at the U.S. National Pollinating Insect Collection. In total, *n* = 24 female specimens were identified. Our goal in assessing historic samples is to identify historic genetic diversity of unmanaged populations. The specimens were collected from Cache and Millard Counties in Utah and Malheur County in Oregon (Table 2) between 1930 and 1960. We aim to use DNA extraction methods that are highly sensitive to the challenges of ancient DNA.

Microsatellite development. To identify microsatellite loci, we surveyed the publicly available annotated genome assembly of the alkali bee (USU_Nmel_1.3), available on NCBI RefSeq (https://www.ncbi.nlm.nih.gov/assembly/GCF_003710045.2) (Kapheim et al. 2019) using Krait (v1.3.3; Du et al. 2018). We queried the genome assembly for imperfect microsatellite markers across 95,883 scaffolds and developed primers to be tested using PCR amplification. Microsatellites were filtered using custom scripts in the R programming language (R Core Team 2020). Of the loci that fit our search criteria, we used a random number generator to randomly select 35 loci for further testing with molecular methods. The loci are provided in Table 3.

Results and next steps

With a team of Utah State University undergraduate lab technicians, we extracted DNA from 338 samples. To date, 35 novel microsatellite markers were amplified using PCR and 17 markers were visually confirmed with gel electrophoresis. Of the 17 markers, we identified 15 markers to reliably amplify in 6 specimens (88.2% success). An example of evidence for amplification of the tested markers are presented in Figure 2. The next steps in our research will be to complete the genotyping with 35 novel microsatellites and conduct analyses to achieve the goals for Objectives 2 and 3. We aim to complete this research by May 2023 and will provide an updated report of our research. Additional funding for this project would support expansion of collecting tissue from unmanaged alkali bees for genetic analysis throughout western North America. Increasing the sampling effort for genetic analysis would enable us to identify wild populations that may be useful for breeding programs and support sustainable managed populations in TGL.

Products

* = undergraduate student

Meredith E.*, Saarman, N. Koch J. Willardson S. *, Jenkins E. *, Burdiss M. *, Seeley T. * 2022. Inbreeding as a source Alkali bee population decline. Utah State University Undergraduate Research Symposium. Utah State University, Logan, UT. (Poster) (Figure 3)

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3. R Core Team. 2020. R Foundation for Statistical Computing, Vienna, Austria.

Figure 2. Novel microsatellite marker NoMe0206C amplifies a fragment of ~275 nucleotides in six alkali bee specimens. Bee sample 4 shows a very light band, likely due to poor DNA quality.

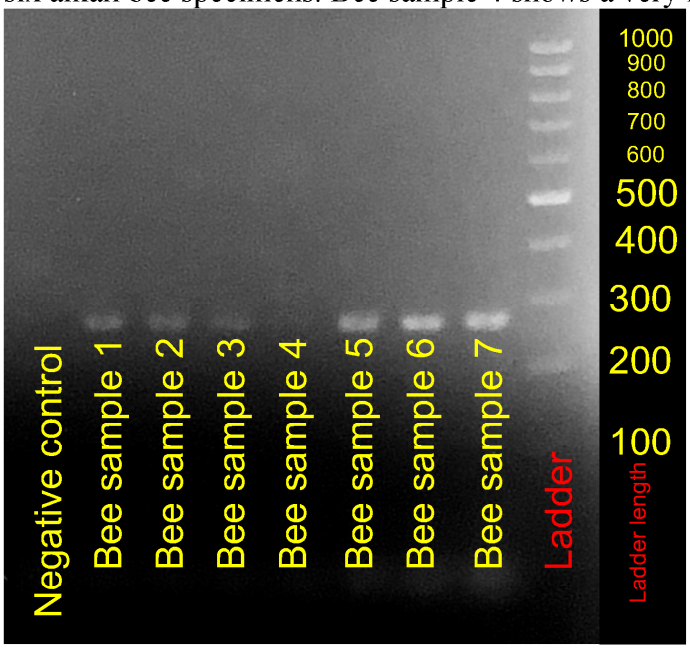

Figure 3. Utah State University undergraduate student Ethan Meredith (right) presents his alkali bee genetics project at an undergraduate research symposium in December 2022. PI Koch (left).

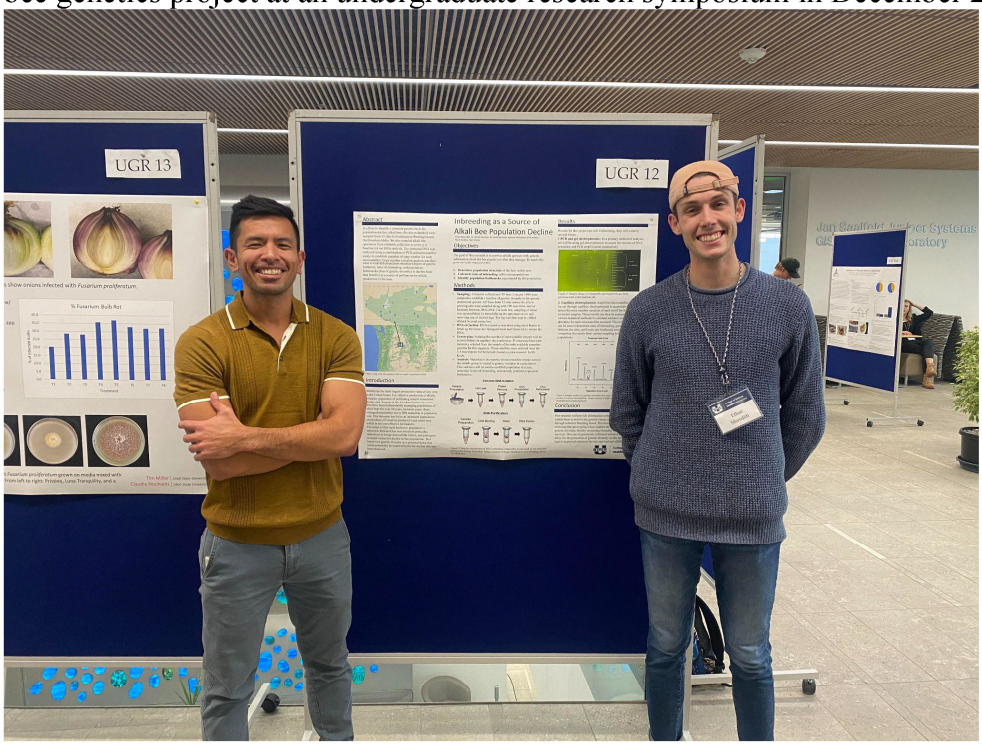

Table 2. Historic alkali bee specimen information. Specimens are housed at the U.S. National Pollinating Insect Collection.

Barcode	Date	Latitude	Longitude	Location	State	County	Elev. (m)
71085	22-Jul-47	41.8383	-111.8319	Smithfield	Utah	Cache	1409
71089	20-Jul-44	41.8383	-111.8319	Smithfield	Utah	Cache	1409
71092	20-Jul-44	41.8383	-111.8319	Smithfield	Utah	Cache	1409
71093	20-Jul-44	41.8383	-111.8319	Smithfield	Utah	Cache	1409
71095	23-Jul-44	41.8383	-111.8319	Smithfield	Utah	Cache	1409
71096	23-Jul-44	41.8383	-111.8319	Smithfield	Utah	Cache	1409
71097	9-Aug-44	41.8383	-111.8319	Smithfield	Utah	Cache	1409
71098	9-Aug-44	41.8383	-111.8319	Smithfield	Utah	Cache	1409
117583	20-Aug-60	43.87	-116.99	Nyssa	Oregon	Malheur	666
117586	27-Jul-61	43.65	-117.06	Adrian, 8 mi S	Oregon	Malheur	710
117588	18-Aug-58	43.8	-117.05	Adrian, 5 mi NW	Oregon	Malheur	670
117589	28-Jun-57	43.66	-117.07	Adrian, 6 mi S	Oregon	Malheur	689
117590	28-Jun-57	43.66	-117.07	Adrian, 6 mi S	Oregon	Malheur	689
117591	28-Jun-57	43.66	-117.07	Adrian, 6 mi S	Oregon	Malheur	689
117592	28-Jun-57	43.66	-117.07	Adrian, 6 mi S	Oregon	Malheur	689
117593	28-Jun-57	43.66	-117.07	Adrian, 6 mi S	Oregon	Malheur	689
117690	31-Jul-49	39.35	-112.57	Delta	Utah	Millard	1414
117500	15-Aug-52	39.35	-112.57	Delta	Utah	Millard	1414
117501	28-Aug-48	39.35	-112.57	Delta	Utah	Millard	1414
117502	30-Jul-48	39.35	-112.57	Delta	Utah	Millard	1414
117503	30-Jul-48	39.35	-112.57	Delta	Utah	Millard	1414
117504	19-Jul-46	39.35	-112.57	Delta	Utah	Millard	1414
117508	15-Aug-52	39.35	-112.57	Delta	Utah	Millard	1414
117694	12-Jul-30	39.35	-112.57	Delta	Utah	Millard	1414

Table 3. Novel microsatellite markers for the alkali bee, *Nomia melenderi*. Primer Name, Motif = motif of microsatellite, Product = expected product size, Forward = forward primer, Reverse = reverse primer.

Primer name	Motif	Product	Forward	Reverse
NoMe0025a	CTCG	202	CACATAATATCCCGGCTACC	TGGCTGATCATAGGATAATGG
NoMe0031a	AATA	196	ATCAAAAGTTCCGGGTAGC	TTCCGAGGTTCGAAATTCC
NoMe0031b	TGC	233	GGTATGATGTTGGGGTACG	TCCCTTTATGGTATGAACGG
NoMe0033a	TATT	255	CTATACTCTCGCTGCTTCC	GAGTGCAGATACCATCTCG
NoMe0033b	AAG	324	ACAACTGAGAAACATGGGG	TCTCTTCCTTGTCTTTGCC
NoMe0058a	ACG	257	GAGGATCGATCAGAAGTCG	TGTGTCTTCTCCTCAGAGG
NoMe0086a	ATT	332	AAGCTGAGGAAGAAAAGAGG	TACACAACCGAAAGAAAAGC
NoMe0086b	AGA	301	AGATTAACTATCGCGAGACG	ATTAATTAAACACGCTGCCG
NoMe0108a	TATG	211	GGTAACAGATTCAAGTTGCC	TTCAAAGAATGAAGGAGGGG
NoMe0119a	TTA	192	GCAACAAAGCGTAGAATCG	TTGAGGAGTAATGGAAGTGC
NoMe0131a	TTC	252	AAAGCATCCCCTAAAGATGG	ACATCTCGACCTATCTACCC
NoMe0150a	TAA	156	AATAACACCCACTAACCTGC	AGGTATATGTAACGTGTGGC
NoMe0150b	AATA	299	GCATGTTTAAGCCTTTCCG	ATGCTATCGGGAACTAAAGG
NoMe0150c	TAAA	255	AAAATCGAGAACACTTTGGC	GACAAAGAGTCCCCTTACG
NoMe0177a	TGA	287	ACAAAGGAACAAGAAATCGC	GTGGTACCTGGTTATGATCG
NoMe0192a	AAT	265	AAAACTTGTTTCGTCGTTCC	GTCTGGTAAGCTCCTATTCG
NoMe0202a	CTTT	167	GAAACTCACATTCGTGTTCG	AAAACAAGAGAGAGGGAAGG
NoMe0206a	TATT	166	GGGTACGTTTCATTGATATCG	TATGCACTGCAATTTTCTGG
NoMe0206b	CGT	225	CTGGTTTAGCATCTATCGGG	CAGTTGGAACGTTTTGTACC
NoMe0206c	TAAA	275	ACAGTATTACGCCTTTACGG	CTACCAAATTTTGCCTACGC
NoMe0302a	AAG	335	TAAATGCGAGGTTTTGTTGG	AACTTGAGTCTCTTCGTACC
NoMe0308a	ACT	172	TGCTTCAGATATTCTAGATGCC	ACTGCAGTGTGACAATGG
NoMe0308b	CATT	207	AGCCATAAATTAGACCTGCC	TGTTAAAAGTTGCAACGAGG
NoMe0308c	TAAG	256	AGAATCCTGCAGTATTACCG	AGTTTACGAGCTTTGTAGGG
NoMe0325a	ATA	172	GGCTCGTGGTATTTTATTGC	TATTTCAAAACGGTGATGCG

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NoMe0325b	ATGT	162	TTCCGGAAAGTTCATCTCG	GTATTCATTCTTACGAGCGC
NoMe0325c	CCG	213	ACCTTTCATCAATAGCTCCG	GCGGATGTATGATGAAACG
NoMe0381a	GTTC	170	AGTTCGAACGTCTATAGTAGC	GTACCACCGGATCTTATTGG
NoMe0478a	ACG	204	CTTATCTCTGTCACTGCTCG	TCGTAAAGACATCTGCACC
NoMe0478b	TAA	323	GTGTTTGCTTCTAGAACACC	CGTTGTAGAGTTAATGCACG
NoMe0482a	GGA	218	CCACGGATGAAAAAGAATCG	GTGGAAAAGGAAGAAACTGC
NoMe0576a	ATT	156	GTATTGCATACAGTCTGTGC	TGCATTTTAACTTCAGTCGC
NoMe0639a	TAT	269	TATGAGGATATCAATGCCGG	GGAAAGTGATTATTCGCATCG
NoMe1127a	CGCC	221	AACGTAAGGAACAGCTTCC	GCTAGTTGAGGGAACTGC
NoMe1564a	TCGC	208	GAAATCCAGCTGATCAAAGC	CCTCTTTACACAACCAGGG

8

Project Title: Pathogen and pesticide screening in managed *Nomia melanderi* populations.

Year(s) of Study: 1 year, March 2022 - February 2023.

Lead Investigator / Affiliation:

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Collaborating Investigator(s) / Affiliation(s):

Diana Cox-Foster, Research Leader, USDA-ARS Pollinating Insects – Biology, Management, & Systematics Research Unit, Logan, UT.

Kimberly Hageman, Associate Professor, Utah State University, Department of Chemistry & Biochemistry, Logan, UT.

Hyperlink to research website and/or curriculum vitae: https://www.ars.usda.gov/people-locations/person/?person-id=55990

Introduction and Justification:
Alfalfa seed growers in the Touchet, WA region have managed nesting beds of alkali bees for over 50 years. Growers in this area have higher seed yields than elsewhere, of which they credit alkali bee pollination (Walsh et al., 2017). However, populations of alkali bees, *Nomia melanderi*, have fluctuated greatly over time, and bee bed failures are not completely understood. Possible causes include exposure to pesticides, reductions in available forage (alfalfa acreage), and a build-up of diseases and pathogens. Bed failures represent significant investment losses for growers who construct and maintain them. Therefore, the aim of this project is to screen for potential causes of bed failure, such as a buildup in viral and fungal pathogens and parasites, which could result in loss of brood and abandonment of nesting beds. Additional correlational factors such as pesticide contamination and soil characteristics will be determined.

Research Update:
Objective 1: Screen *Nomia* populations for pathogens and parasites.

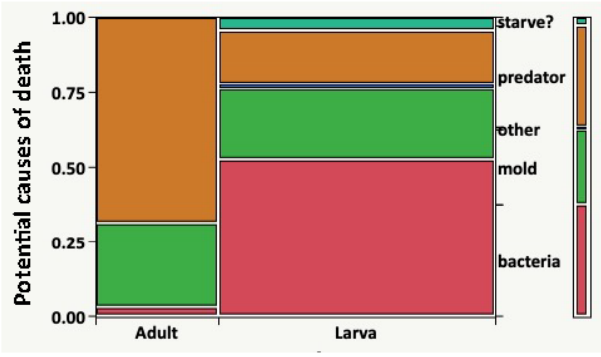
In 2022, nine alkali bee beds near Touchet, WA, were sampled for pathogens, with samples taken in June, some in July, and later in August. The samples in June were taken at the beginning of bee emergence and flights, and enabled sampling the nests from the previous year to determine percent of live vs. dead bees in the bee beds with high density nesting. Samples in July (low density or poor bee beds) and August (all bee beds) were taken to sample larvae from the 2022 season to determine their health status. Samples were taken of both the bees, pollen balls, and other associated arthropods (predators or scavengers). Samples were either directly frozen in liquid nitrogen in microcentrifuge tubes or placed in microwell plates and kept cool. Samples in liquid nitrogen were transferred to -80°C for future molecular analysis. Samples in the microwell plates were brought back to Logan for examination under dissecting microscope.

Soil was also taken to test for the presence of entomopathogenic nematodes; entomopathogenic nematodes infect insects that are soil dwelling or present in the upper layers. Previous reports of experimental infections in bees by entomopathogenic nematodes has been reported for bumble bees. Presence of the nematodes could explain different areas of a bee bed being impacted. In the nine bee beds, soil was taken from areas with high, medium, or low nesting activity; two of the beds had only low nest activity. Soil samples (approx. 50 ml) were kept cool and brought back to Logan. The samples were moistened with sterile tap water and mixed. Four last instar wax moth larvae *(Galleria mellonella)* added to each cup and monitored for nematode infection. A total of 85 soil samples (34 from areas with low nesting activity, 9 from areas of medium nesting activity, and 42 samples from high nesting activity) were evaluated. No entomopathogenic nematodes were detected at either early season or late season.

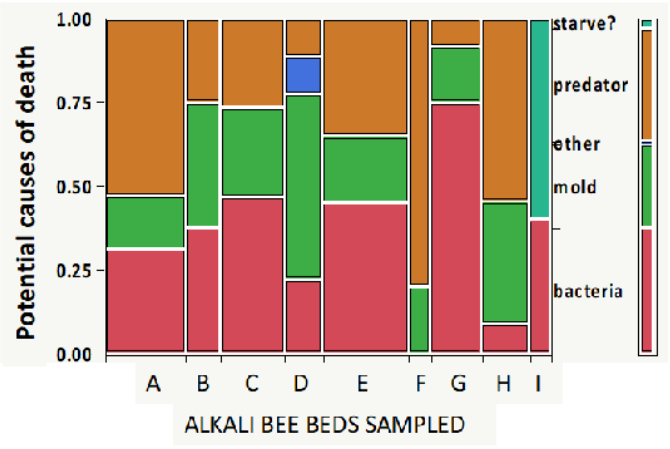
For the other pathogens, 475 samples were collected from the bee beds for molecular analysis. The nine alkali bee beds varied in the density of nesting by the alkali bees, from very low, to medium, to high. Within each bee bed, different regions also varied in nesting density. To sample, one shovel sample (an area of about 10 X 10 X 8 in^3) was dug and nest cells from 5-10 bees nests were carefully excavated. In areas of extremely low nesting, additional shovel samples were taken to get more samples. From the nine bee beds, the sample number ranged from 22/27 for the poor bee beds with low density nesting, to 42 – 64 samples from bee beds with higher nesting density (samples from low, medium, and high density regions within each).

Samples were characterized by stage of development (larvae (all instars/prepupae), adults, pollen pollens, and unknown) and the health status (alive/dead). Other key characters were noted, such as evidence of predation, potential bacterial infection, or fungal/mold infection. In June or at the beginning of bee emergence, dead adults were found in seven of the beds, with evidence of predation; it is not known if the adults from the previous year died in the nests or if a predator consumed them prior to emergence. A significant number of dead larvae were found in early June in the seven bee beds; these bees had evidence of fungal infections. It is not known if these larvae died from the fungi or if the fungi developed on the cadaver. In August, dead larvae were found and symptoms were split between evidence of predation (possibly by bee fly larvae) or a bacterial infection (melanized areas or discolored, soft bodies). There was no significant difference in the percent dead larvae in the bee beds with low nesting versus those with high nesting. Pollen balls were found in early season in bee beds with low density and high density. In low density areas, the pollen balls made up about 18% of all the samples; in high density areas, the pollen balls made up about 13% of the samples. These pollen samples had significant visible mold and bacterial growth.

For the dead adults and larvae in the nests cells, there was a significant difference in the possible causes of death. (Likelihood Ratio Test. ChiSquare =39.363, Prob>ChiSq = <.0001*)



The bee beds significantly differed in the cause of death among the sample adults and larvae (combined analysis of adults and larvae, (Likelihood Ratio Test. ChiSquare =56.670, Prob>ChiSq = 0.0046*). The other cause of death is unknown for the larva; it was opaque white and soft in texture. In one bee bed, dead small larvae were found without pollen, raising the potential that they starved to death; this needs to be investigated more.



Molecular analyses are still progressing to determine the presence of viruses and the identity of bacteria/fungi, with delay due to issues related to COVID and people allowed in the lab. (Anticipated completion June 2023). Previously, samples of alkali bees from the Touchet area that were sent to Rosalind James and analyzed by Rajwinder Singh (Singh, 2011) had four detected viruses: deformed wing virus (DWV), black queen cell virus (BQCV), Israeli acute bee paralysis virus (IAPV), and sacbrood virus (SBV). Additional samples taken later by Karen Kapheim had detections of DWV and BQCV. How the viruses impact the alkali bee is unknown, but environmental stress factors (pesticides and spray adjuvants) may synergize causing greater impact. For the mold or fungal cause of death, known fungal pathogens for the alkali bee include Aspergillus, Mucor, Fusarium, and Ascosphaera species (Batra and Bohart, 1969; Stephen, 1959). Justin Clements had also identified numerous fungal species in the nest cells. The sequences for the pathogens will be informative to indicate if common causes of death exist between the nine different bee beds.

Potentially, additional experiments are needed to determine the identity of the predators of both the adults and the larvae. Controls for these insects or arthropods could potentially be developed.

Objective 2: Quantify residues of lambda-cyhalothrin in *Nomia* bee beds and pollen provisions.

In June 2021, we sampled soil from eight alkali bee beds near Touchet, WA, with a range of nesting activity. At each bed, we used a 14-inch soil sampler to sample at five locations in the bed. Each sample was then separated into top soil (upper 7" of the soil sample) and lower soil (lower 7"). Samples were sent to the Cornell Chemical Ecology Core Facility (CCECF) for residue extraction and analysis of 95 commonly used pesticides, though not lambda-cyhalothrin (Warrior). Additionally, to screen for pesticides in bee provisioned pollen, we dug up completed nests at eight productive bee beds. Pollen provisions were removed and sent to the CCECF for residue analysis, as well. The pesticide results from the CCECF were previously presented to seed growers at the Touchet-Lowden-Gardena Field day in May 2022. For this APRI funding, sample extracts were shipped to Dr. Hageman's lab at Utah State University, who quantified lambda-cyhalothrin in soil and pollen.

Warrior, lambda-cyhalothrin, was detected in one pollen sample and one soil sample (at different farms), but both detections were at very low concentrations. The concentration in the pollen sample represented 0.007% of the honey bee LD50 (oral exposure), and the concentration in the soil sample represented 0.01% of the honey bee LD50 (contact exposure). While this indicates that the exposure pathway exists for alkali bees to come into contact with Warrior, these are not concentrations that would indicate a significant risk to bee health. The most protective threshold of concern for regulatory agencies is 3% of the LD50 for oral exposures.

Objective 3: Characterize the soil at successful and unsuccessful bee beds.

We took soil samples using a 14-inch soil sampler at 9 Nomia bee beds in the Touchet-Gardena-Lowden area of eastern WA in 2021 as described above. Including separating the samples between the upper 7 inches of soil and the lower 7 inches of soil. Three of the bee beds had little to no *Nomia* nesting and had poor nesting in recent years. The other six beds had good *Nomia* nesting. Additionally, in 2022, we used a soil moisture probe to measure percent moisture at the

surface of six bee beds. At each bed, we took measurements where there was early *Nomia* emergence in 2022 and at locations where emergence was later (1-2 weeks after).

Soil samples from 2021 were then characterized at the Utah State University Analytical Laboratory, Logan, UT. Analyses included pH, electrical conductivity, sodium absorption ratio, percent organic matter, texture analysis, and moisture content. Results were then compared between good and bad beds, as well as compared to previous work characterizing the optimal soil conditions for alkali bee beds (Stephen, 1960; Stephen and Evans, 1960).

We compared the soil composition data between bee beds with good and bad nesting, composition was very similar, with the only significant difference between good and bad beds being the average percent of clay in the soil (Mann–Whitney $U = 9$, $n_1 = n_2 = 6$, $p = 0.008$, two-tailed). Beds with good nesting had an average (mean) clay composition of 13.1% ± 0.6 S.E., and bad nesting beds had an average clay composition of 9.9% ± 0.8 S.E. (Fig. 1). This is counter to previous results which suggested that lower clay composition (below 7%) was optimal for *N. melanderi* nesting (Stephen, 1960; Stephen and Evans, 1960). However, the use of surface salts may circumvent issues around increased clay content by increasing capillary action in the soils. In general, most ground-nesting bees prefer soils with low clay, high sand, and high silt contents (Cane, 1991), though there are also published accounts of bees nesting in soils in the Willamette Valley of Oregon that had average clay contents well above 20% (Lybrand et al., 2020). Higher levels of clay do not seem to impede *N. melanderi* nesting under the right circumstances.

We also compared the soil composition data between the upper 7 inches of soil and the bottom 7 inches of soil at beds with good nesting. It's previously been recorded that the average *N. melanderi* nest is between 5 and 10 inches deep (Bohart, 1950; Bohart and Cross, 1955). Sodium absorption ratio (SAR), electrical conductivity (EC) and percent moisture were all significantly different between the upper and lower layers of the soil (Fig. 2). SAR is the measure of the amount of sodium relative to calcium and magnesium, and an SAR greater than 13 is considered soil with excessive levels of sodium absorbed (Soil Health Nexus, 2020a). EC is a measure of the concentration of ions from water-soluble salts and is also representative of soil salinity (Soil Health Nexus, 2020b). An EC >4 dS/m would be considered saline soil. High SAR (>13) combined with low EC (<2 dS/m) can indicate that movement of soil water is restricted (Soil Health Nexus, 2020a). SAR and EC were both significantly lower in the bottom 7 inches (SAR: 72.0 ± 12.1 S.E.; EC: 29.1 dS/m ± 5.9 S.E.), compared to the top 7 inches (SAR: 127.8 ± 17.0 S.E.; EC: 75.2 dS/m ± 6.0 S.E.), though both were generally high and indicate highly saline soils. Moisture was significantly higher in the bottom 7 inches (16.2% ± 0.5 S.E.) compared to the top 7 inches (13.8% ± 0.5 S.E.). Previous work has shown that maintaining soil moisture is key to the long term success of *N. melanderi* nests (Stephen and Evans, 1960), and surface moisture levels around 12% were recorded for excellent bee beds (Stephen, 1960).

We also wanted to see if differences in surface moisture could be driving the timing of initial *Nomia melanderi* emergence. We found a trend towards earlier emerging bees being in areas of beds with higher surface moisture content (LMER: $X^2 = 3.61$, df = 1, $p = 0.057$), but the difference was not significant (Fig. 3). Elevated soil moisture has previously been thought to delay bee emergence (Hackwell, 1967), so this trend would be counter to prior assumptions. However, all locations had high surface moisture levels in 2022 (generally greater than 40% moisture). Spring moisture levels have been recorded previously between 30-35% moisture, dropping to around 20-25% moisture during bee emergence (Stephen, 1959). This was an unusually cold, wet spring which may have led to unusually high moisture content in the soil of

bee beds early in the season, and therefore may have impacted our ability to detect how soil moisture impacts emergence timing.

Taken together, I don't see any of the tested soil characteristics being a clear sign of good or bad nesting activity for alkali bees, *Nomia melanderi*. Soil characteristics were very similar between good and bad beds, with only percent clay being significantly different between them, though in both cases the average percent clay was higher than has been previously assessed as an optimal content. Other indicators of moisture movement in the soils seem good, including SAR, EC and percent moisture, with differences between the top and bottom layers of soil, but nothing to indicate a potential problem with the movement of water in the soil.

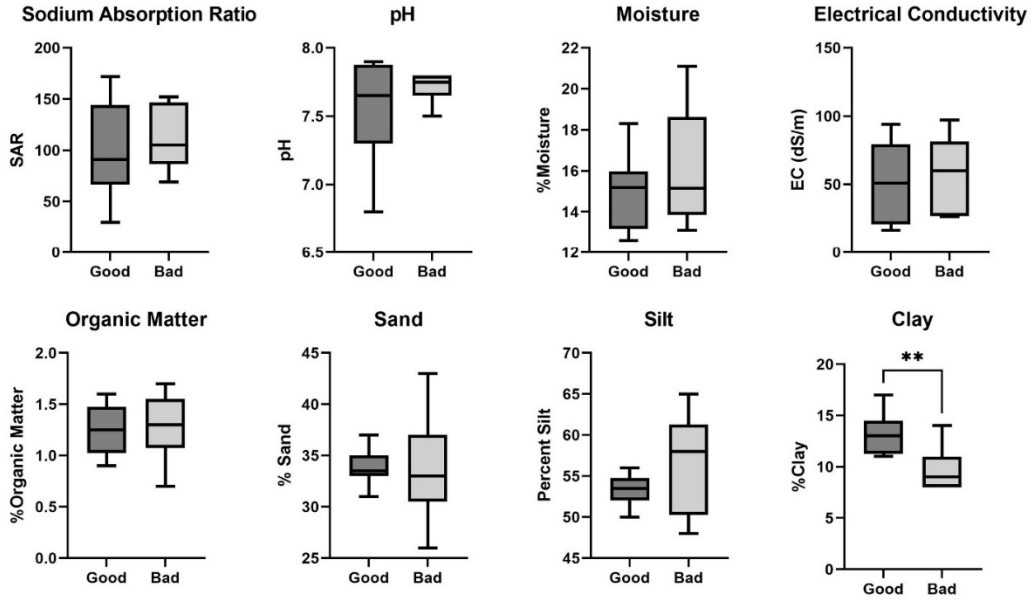


Figure 1. Comparison of soil composition results between Nomia bee beds that had good nesting ("Good") and bad nesting ("Bad"). The only factor that was significantly different between the bed categories was percent of clay.

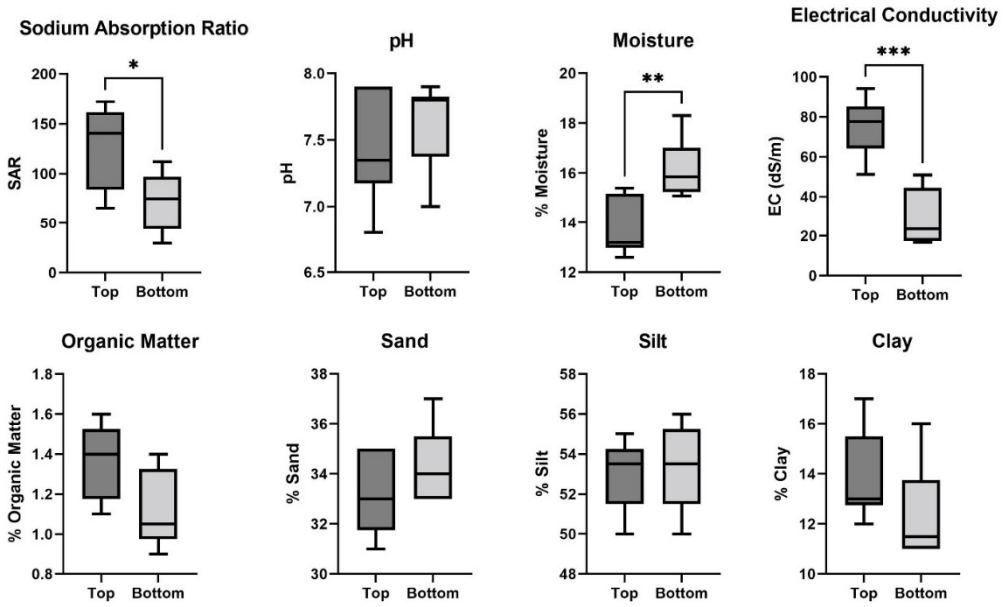


Figure 2. Comparison of soil composition results between the top 7 inches of soil ("Top") and the bottom 7 inches of soil ("Bottom") at beds with good nesting. Sodium Absorption Ratio, Moisture, and Electrical Conductivity were all significant.

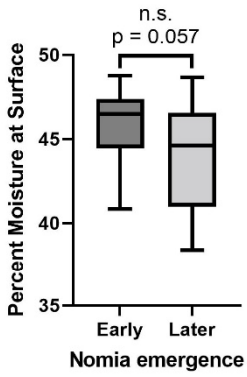


Figure 3. Average percent moisture at the surface of six bee beds in areas of early or later (1-2 weeks later) *Nomia melanderi* emergence in 2022.

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Project Title: Assessment of seasonal and yearly variability of alfalfa leafcutting bee (*Megachile rotundata*) parasite infestation in seed production fields

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Introduction: Natural enemies are one of the leading causes of mortality in alfalfa leafcutting bee (ALCB: *Megachile rotundata*) populations in the western United States. Most enemies are parasites and parasitoids, including *Monodontomerus*, *Pteromalus*, *Tetrastichus Melittobia*, Meloidae, and *Sapyga*. These different insects can all have detrimental effects on unique life stages of ALCB populations (Pitts-Singer and Cane 2011); furthermore, some of these pests do not have recommended control measurements. Levels of even 1% parasitism can have determinantal economic impacts (Eves et al. 1980). While we know the general life history of these insects, what is lacking is the seasonal timing in which they infest the ALCB nests in the field. Understanding the pest life history and timing of activity in the field is important for managing these species.

Environmental conditions such as temperature and humidity can affect the reproductive success of ALCBs, mainly in the increased mortality of the immature stages as well as increased incidence of pollen ball cells (Pitts-Singer and James 2008; James and Pitts-Singer 2013). However, what is relatively unknown is how these different environmental conditions could impact the infestation rates and timing in ALCB populations during the nesting season, rather than changes in temperature during developmental periods. Here we will examine if timing and environmental conditions correlate with parasite and parastioid occurrence rates under field conditions where ALCB commercial populations nest in dense numbers.

Objective(s): Our project addresses the seasonality of pest infestations in a northern Utah commercial alfalfa field in a two-year study.

Methods: In Year 1, we worked with two alfalfa growers in Trenton, UT and in Malad City, ID who released ALCBs at normal stocking density (4-5 gallons of bees per acre). Shelters with parts of polystyrene bee boards having paper straws inserted into nesting holes were provided for bees. Each week, for a total of nine weeks, from the release of bees to the end of alfalfa bloom (June– August), we collected a sample of nests. We sampled from three domiciles at each location (180 -540 straws each week). Within each domicile, four board faces were sampled. On each board face we inserted a diagonal transect of straws in three sections of the board, roughly 30 straws per section. The straws in which nests were made were pulled once a week and brought back to the lab to monitor development of bees or pests within. Additional nests were also brought back after being in the field for two and three weeks in order to capture the parasitoids that use mature larvae as hosts. These diagonal transects were arranged so that we can test nest placement in relationship to where infestations take place. We examined the nesting straws collected weekly; nesting straws from these fields were x-rayed every three days to look at parasitism, pollen balls, chalkbrood, and second generation bees. Additionally, HOBO dataloggers were placed in each of the 10 boards to correlate temperature and humidity with infestation throughout the nesting season.

Results and Discussion: We found that weather events predicted the occurrence of three pests in two farms. Both sites responded to these weather conditions in the same predictable manner regarding pest occurrences ($t = -0.475$, $p = 0.649$). In the samples we took, we found the two kleptoparasites: a meloid beetle and the wasp *Sapyga.* We found two parasitoids: *Pteromalus*

and *Melittobia*. We also found the predator *Trichodes. Pteromalus* and Meloidae were not detected at levels that could be included in this Year 1 analysis.

We found that *Melittobia* occurrences were predicted by mean temperature during alfalfa bloom. When mean temperature was low, *Melittobia* numbers were the greatest ($t = -2.271$, $p = 0.040$, Figure 1). Minimum relative humidity was also marginally significant, where slightly higher *Melittobia* incidents were detected with higher relative humidity ($z = 1.819$, $p = 0.092$). There was no interaction effect between mean temperature and minimum humidity.

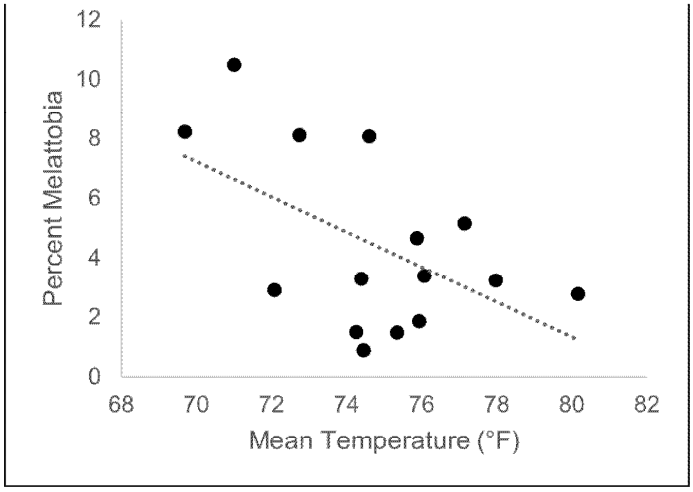

Figure 1: Percent *Melittobia* cells to total ALCB cells in relation to mean temperature. Dotted trend line denotes significance.

Sapyga incidents were correlated with both temperature and humidity. As maximum temperatures increased so did number of parasitized cells by *Sapyga* ($z = -1.839$, $p = 0.053$, Figure 2A). Additionally minimum relative humidity predicted the number of parasitized *Sapyga*

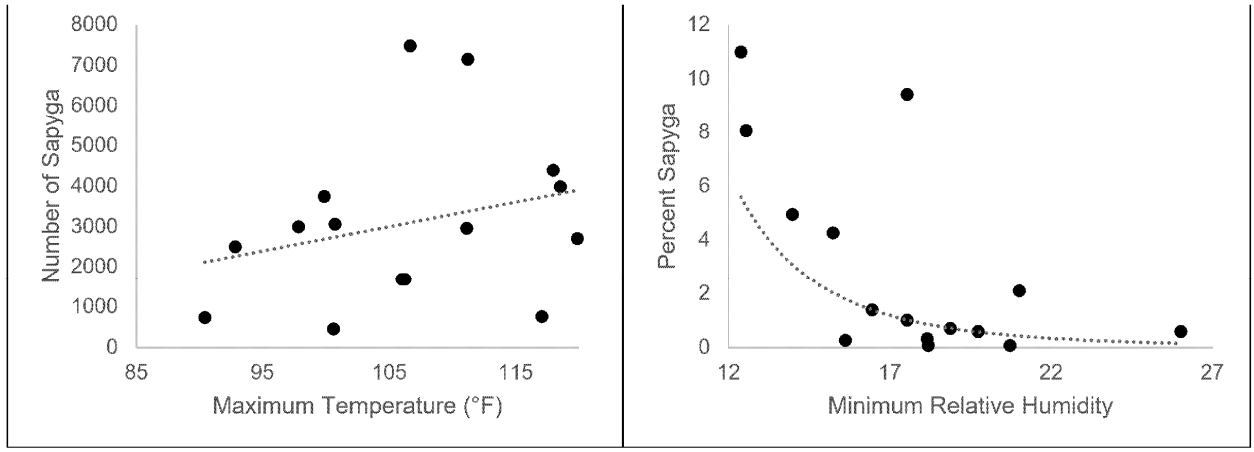

Figure 2: (A) Number of *Sapyga* in relationship to maximum temperature. (B) Percent *Sapyga* cells to total ALCB cells in relationship to minimum relative humidity. Dotted trend lines denote significance.

cells; when humidity was low, *Sapyga* infestation was the greatest (z = -2.859, p = 0.013, Figure 2B). The interaction between maximum temperature and minimum relative humidity was the greatest predictor of *Sapyga* incidents (t = 1.225, p = 0.024). This means that during hot dry times of the season, there is a greater probability of *Sapyga* parasitizing ALCB.

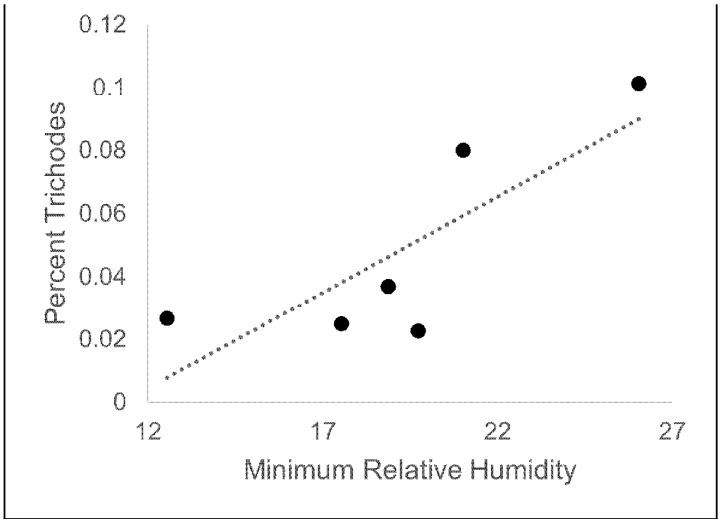

Figure 3: Percent *Trichodes* cells to total ALCB cells in relation to minimum relative humidity. Dotted trend line denotes significance.

Finally, we found that *Trichodes* incidents were predicted by minimum relative humidity; as minimum relative humidity increased so did *Trichodes* incidents (t = 5.484, p = 0.047, Figure 3). None of the parasites had a relationship with mean or maximum humidity nor minimum temperatures.

Conclusions: Extreme weather and climate events can dictate how pestiferous insects respond to their environment and, therefore, when they infest ALCBs. This information will provide base line information to help develop models that can predict when ALCBs are susceptible to pest attacks and potentially can inform accurate mitigation measures. We will continue this project in 2023, collecting more data to create a more robust model.

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Enhancing and Protecting Populations of Alfalfa Seed Pollinators - 2022

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Introduction:

Pollination by alfalfa leafcutting bees *(Megachile rotundata,* ALCB) or alkali bees *(Nomia melanderi,* AB) is essential for seed set in alfalfa seed production. Bee mortality that results from inadvertent exposure to pesticides can negatively impact bee survival and/or fitness and potentially reduce seed yields. The first objective of our 2022 studies involved trying to use thermal image technology to monitor leafcutting bee behaviors in and on bee boards. We tried up-close analysis of research bee boards with a thermal imager under controlled laboratory conditions. The binder boards had been left in the domiciles for 1 week and subsequently they were sealed and transported back to WSU IAREC. In the lab, under caged conditions, we tried to take images of bees as they emerged from the binder boards. We got no thermal images of these bees. Only a few bees emerged from their nest holes and none of them went back to the binder board as they struggled to escape from the cage in which the binder boards were contained. Our second objective involved facilitating the registration of insecticides for the key pests of alfalfa produced for seed during bloom by testing the effects of ALCB to direct topical exposure. In 2022 we focused on one candidate insecticide that has been registered on other crops in recent years for homopteran insect suppression and control and on a second proprietary insecticide that is well into the registration process on multiple crops. Both of these insecticides would be considered safe for leafcutting bees at the rates we tested. In our time-tested contact bioassays mortality of ALCB was 13% for the proprietary insecticide and 4% for afidopyropen. For our third objective we conducted our annual survey of alkali bees in Touchet-Lowden alfalfa seed growing district. Unfortunately, the population abundance of alkali bees had been declining over time as the alfalfa seed production in the area declined. Fortunately, we did quantify a bump up in the population abundance of alkali bees in 2022 compared to 2021. Our fourth objective was not completed due to the separation of molecular toxicologist and entomologist Dr. Justin Clements from the University of Idaho. Dr. Clements and his team had developed multiplex molecular methodology for identifying and quantifying pathogen and parasite infestations in ALCB (Clements et al. 2022). We had planned on expanding this methodology to include brood from endemic alkali and metallic sweat bees in 2022.

Objectives:

Objective 1. Use thermal imagery technology to quantify foraging behavior in general and compare behaviors exhibited by bees exposed to sulfoxaflor compared to unexposed bees. We tried to complete some controlled lab studies with this technology and frankly, it did not work. We placed 3 research binder boards in ALCB domiciles near Lowden WA on June 13, 2022. We left the boards in the domiciles for 1 week then sealed them with duct tape and transported them back to the lab at WSU IAREC where we placed them in Bioquip™ cages and removed the duct tape. Among the 3 binder boards in cage only 5 ALCB emerged in total and none of these bees ever went back to the binder board. Cotton balls imbibed with a sucrose solution was provided in a Petri dish in the cage. None of the bees were observed feeding on the cotton balls. All of the bees perished in about 2 days. The experiment failed.

Objective 2. Conduct topical treatment tests on alfalfa leafcutting bees with a proprietary chemistry and afidopyropen. In 2022 we focused on a proprietary candidate insecticide and a newer, reduced-risk insecticide, afidopyropen. Both insecticides were applied with a R&D CO_2 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 8, 2022. Field-weathered residual test exposures on each insecticide were replicated 5 times per candidate insecticide at 1 hour after the insecticides were applied. Samples consisting of cutting approximately 400 cm^3 of foliage 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) to create a cage.

Extant ALCB were collected by sweep net from alfalfa fields grown for seed at the entrance of ALCB domiciles. The bees were tranquilized with CO_2 and put in the Petri dish bioassay cages. 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 conclusive that the proprietary chemistry and afidopyropen (Sefina) could be considered safe for foraging 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 to 8 hrs of exposure to treated alfalfa foliage collected 1 hr after insecticide application.

Product	Rate per acre	ALCB % Corrected Mortality
Proprietary	81 g	13%
Afidopyropen (Sefina)	14 oz	4%

Objective 3. Conduct an annual census of the alkali bee population abundance in Walla Walla County, WA (2010 – 2022). Alkali bee emergence hole counts have been recorded annually at the end of the alkali bee nesting season (mid to late July) from 2010 to 2022, in accordance with standardized methods established by Vinchesi and Walsh in 2014. In this method, 0.5m^2 quadrats made of lightweight PVC pipe, with dimensions of 0.7m by 0.7m, are tossed randomly across each surveyed bee bed 24 times, and the number of emergence holes contained within each quadrat is counted and recorded. The same 13 bee beds were consistently sampled year-after-year and were initially selected for observation due to known history of alkali bee nesting activity, ease of access, and interest from grower collaborators. In 2021 Bed 12 was eliminated from the study because the grower had abandoned it and the bed was condemned by the Washington Department of Transportation since it is in the roadbed of the new Highway 12 upgrade project connecting Wallula to Frenchtown. New beds were added to the survey in 2018 and added to the total count of bees but for consistency we report these data as a separate value and leave a column in Table 2 to represent the original 13 (now 12) beds. At each bee bed, special care was taken to ensure that each quadrat landed in a previously unsampled space.

Surveyors "calibrated" their counts at the beginning of the survey by counting three quadrats together to ensure that each person counted the same number of bee emergence holes. For continuity, every year at least one of the surveyors had participated in surveys the prior year. In 2022 all 3 surveyors had multiple years of experience. The emergence hole counts were used to estimate the number of active nests per bee bed using the following formula:

2 x ([Mean number of quadrat counts per 0.5 m2 ± SE] x [2/3] x [surface area of bee bed])

This formula was first proposed by Jim Cane through video observations of nesting activity that found that two-thirds of nest holes were being actively provisioned. The practice of using surface nest holes to estimate alkali bee populations was then validated by Vinchesi and Walsh (2014), which confirmed that surface nest hole counts were tightly correlated with the abundance of belowground prepupae. The above formula has been adjusted to rectify an error in Vinchesi and Walsh in 2014, which failed to account for the use of $0.5m^2$ quadrats instead of $1m^2$ quadrats. As a result, all population estimates previously reported by Vinchesi and Walsh in 2014 were doubled before inclusion here.

Results

From 2010 to 2021 the estimated population abundance of alkali bees varied from a low in 2021 of 1.6 million from the peak of over 9.4 million in 2012 (Table 2). These results have been alarming and it is unfortunate that the alkali bee population dropped so dramatically over that period of time. However, the addition of 3 fairly new and very active bee beds brought the total count to 2.5 million in 2022.

Table 2. Estimated population abundance of alkali bees from 13 managed bee beds from 2010 through 2017, in 16 managed bee beds from 2018 through 2020, and in 15 managed beds in 2022 in the Touchet Valley of Walla Walla County, WA. Bed 12 from the original 13 was eliminated in 2021.

	Original 13 beds	Plus 3 new beds
2010	8,437,000	
2011	5,335,000	
2012	9,428,000	
2013	6,917,000	
2014	4,005,000	
2015	6,177,000	
2016	8,211,000	
2017	7,053,000	
2018	7,354,000	10,294,000
2019	4,763,000	6,550,000
2020	3,590,000	4,564,000
2021	1,601,921	2,505,126
2022	2,279,528	3,272,243

Discussion

Alkali bees continue to serve as an important resource for alfalfa seed growers in the Touchet, Gardena, and Lowden alfalfa seed growing areas. The population abundance of these bees has dropped over several years. Economic issues and low demand for seed led to a decrease in acreage over the past several years. This may have contributed to declines in alkali bees. We anticipate that 2023 will be another low year for alkali bee abundance. However, we had one event in 2014 in which an individual grower had a mishap and treated their fields with their late spring clean-up spray in 2013. This single event led to a dramatic drop in the total abundance of bees in 2014 to just over 4 million bees. However, the bee abundance in this bed recovered to its original population abundance by 2015. Well managed alkali bee beds appear to be very resilient. If economic conditions improve for alfalfa seed growers, we anticipate that alkali bees will prove resilient and rebound.

Objective 4. Collect alkali bees, ALCB, and *Agapostemon* spp. adults, larvae, and pupae for analysis of the various pathogens that may be shared among these bee species. Dr. Justin Clements, formerly with the University of Idaho, had developed a multiplex method to assess the biodiversity of organisms that plague ALCB (Clements et al. 2022). We had planned on collecting ground-dwelling larvae and pupae of alkali bees and metallic sweat bees from in and near fields of alfalfa grown for seed in and near fields of alfalfa grown for seed in the Touchet-Lowden, WA alfalfa growing district. Dr. Clements separated from the University of Idaho and unfortunately this left a large gap in our capacity. The funding level provided by this grant was insufficient to bring another molecular toxicologist into this project. This work was not completed in 2022.

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Lygus and Weevil Management on Alfalfa Produced for Seed

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Project Summary: Washington State alfalfa seed growers must control their key crop-limiting pest Lygus bug. Economic control of Lygus requires insecticide applications pre-bloom and during bloom, which unfortunately can coincide with the timeframe during which pollinators can be actively foraging and are provisioning their brood, rendering themselves and the next generation of bees vulnerable to insecticide exposure. This limits the cohort of insecticides available for use during bloom. Hence the need for additional testing. The revocation of the tolerance for chlorpyrifos on alfalfa impacts alfalfa seed growers and will only serve to increase overreliance on synthetic pyrethroids for alfalfa weevil control. Hence we have take a snap-shot on the resistance status of alfalfa weevils in several forage and seed growing areas of Washington State to several commonly applied pyrethroid insecticides.

Project Objectives:

a) Conduct insecticide efficacy tests for candidate Lygus control insecticides for use in the pre-bloom cleanup program.

b) Conduct insecticide efficacy tests for candidate Lygus control insecticides during bloom.

c) Conduct Bioassays to quantify resistance status of alfalfa weevil populations to pyrethroid insecticides.

Progress by objective:

Objective a) Conduct insecticide efficacy tests for candidate Lygus control insecticides for use in the pre-bloom cleanup program. Table 1 details the treatments in our pre-bloom 2022 spray program.

Table 1. Detail of Lygus/aphid pre-bloom sprays on Alfalfa Seed 2022

#	Product	Active ingredient	Rate/acre	
1	Untreated Control			
2	Beleaf 50 SG	flonicamid	2.8	oz/acre
3	Brigade 2EC	bifenthrin	6.4	Fl oz/acre
4	Proprietary	propriatary	81	g/acre
5	Mustang Max	zeta-cypermethrin	4	Fl oz/acre
6	Orthene 97	acephate	1	lb/acre
7	Transform	sulfoxaflor	2.25	oz/acre
8	Steward	Indoxcarb	11.3	Fl oz/acre
9	Sefina	afidopyropen	10	Fl oz/acre
10	Sefina	afidopyropen	14	Fl oz/acre
11	Brigade 2EC	bifenthrin	6.4	Fl oz/acre
	plus Mustang Max	zeta-cypermethrin	4	Fl oz/acre

Insecticides were applied on 6/22/2022 to 4 replicate plots of 12' by 20' per treatment by CO2 powered flat fan boom sprayer. Plots were sampled pre-treatment on 6/21/2022 and 2, 7, 9, and 15 days after treatment on 6/24, 6/29, 7/1, and 7/7/2022, respectively. Sampling was by five 180° sweeps per plot and counting the number of pests including adult Lygus, large and small lygus nymphs, aphids, and alfalfa weevils, and beneficial arthropods including spiders, big-eyed bugs, minute pirate bugs, nabids, lady bird beetles, and lace wing larva capture within the net. Data was entered in spreadsheets. Data was analyzed by analysis of variance and if treatment difference were significant ($p<0.05$) pairwise *t*-tests we completed between the untreated control and each respective insecticide treatment. There were no statistical differences in the abundance of beneficials and were usually low among the samples collected and the data is not shown.

Table 2. Pest abundance as adult Lygus and large and small Lygus nymphs and aphids per five 180° sweeps with a sweep net.

1-day Pretreatment

21-Jun Treatment	rate	Lygus Adults	lrg Nymphs	Sml Nymphs	Aphids
Beleaf 50 SG	2.8 oz	15.25	4.50	1.00	95.00
Brigade & Mustang	6.4 & 4 oz	14.50	5.50	1.50	95.00
Brigade 2 EC	6.4 oz	16.50	5.25	3.25	82.50
Mustang Max	4 oz	18.25	6.25	0.00	110.00
Orthene 97	1 lb	16.50	6.00	2.00	132.50
Sefina 10	10 oz	14.75	3.00	1.00	110.00
Sefina 14	14 oz	10.00	5.25	4.75	100.00
Proprietary	81 g	13.25	7.25	1.00	125.00
Steward	11.3 oz	15.50	6.25	1.50	85.00
Transform	2.25 oz	14.00	4.50	2.50	132.50
Untreated	0	11.00	4.75	1.25	107.50

2 days after treatment

24-Jun Treament	rate	Lygus Adults	lrg Nymps	Sml Nymphs	Aphids
Beleaf 50 SG	2.8 oz	10.5**	1.00**	0.00**	38.25**
Brigade & Mustang	6.4 & 4 oz	7.75**	0.25**	0.00**	2.25**
Brigade 2 EC	6.4 oz	8.00**	2.00**	1.25**	3.25**
Mustang Max	4 oz	8.00**	1.25**	1.75**	47.75*
Orthene 97	1 lb	13.50**	1.25**	0.75**	41.50*
Sefina 10	10 oz	9.75**	2.75*	1.50**	23.25**
Sefina 14	14 oz	19.00ns	1.75**	1.75**	18.25**
Proprietary	81 g	26.50ns	4.25ns	2.25ns	56.50ns
Steward	11.3 oz	14.00**	0.50**	0.75**	60.25ns

Transform	2.25 oz	12.50.**	1.25**	0.25**	28.00**
Untreated	0	29.75	5.75	4.25	103.75

7 days after treatment

29-Jun		Lygus			
Treament	rate	Adults	lrg Nymps	Sml Nymphs	Aphids
Beleaf 50 SG	2.8 oz	27.25**	3.00*	12.25ns	45.00ns
Brigade & Mustang	6.4 & 4 oz	20.50**	1.00*	1.75**	11.50**
Brigade 2 EC	6.4 oz	20.75**	1.00*	6.25**	3.50**
Mustang Max	4 oz	20.50**	1.00*	9.00*	6.50**
Orthene 97	1 lb	27.75**	2.00*	5.00**	60.00ns
Sefina 10	10 oz	23.25**	2.75*	17.00ns	5.50**
Sefina 14	14 oz	26.50**	4.75ns	21.75ns	4.50**
Proprietary	81 g	34.50ns	3.75*	15.50ns	25.00ns
Steward	11.3 oz	34.25ns	1.50*	10.00*	78.75ns
Transform	2.25 oz	39.50ns	2.50*	6.00**	41.00ns
Untreated	0	46.00	6.00	15.50	45.75ns

9 days after treatment

1-Jul		Lygus			
Treament	rate	Adults	lrg Nymps	Sml Nymphs	Aphids
Beleaf 50 SG	2.8 oz	22.25	3.25*	5	34
Brigade & Mustang	6.4 & 4 oz	25	0.25*	2.5	3.25
Brigade 2 EC	6.4 oz	23.75	1.50*	4.75	1.25
Mustang Max	4 oz	22	4.00*	11	36
Orthene 97	1 lb	22.5	1.25*	6	15
Sefina 10	10 oz	27.5	4.75*	15.75	8.75
Sefina 14	14 oz	15.75	1.75*	18.25	10
Propriatory	81 g	29.25	6.25ns	14.25	17
Steward	11.3 oz	28.25	0.75*	9	63.25
Transform	2.25 oz	25	3.50ns	9	8.75
Untreated	0	24.5	4	13.5	47.25

15 days after treatment

7-Jul		Lygus			
Treament	rate	Adults	lrg Nymps	Sml Nymphs	Aphids
Beleaf 50 SG	2.8 oz	26.75	5.25ns	18.75	3
Brigade & Mustang	6.4 & 4 oz	31	3.75*	14.25	0.75
Brigade 2 EC	6.4 oz	26.25	8.25ns	21.25	2.75
Mustang Max	4 oz	20	9.25ns	39.5	6.25
Orthene 97	1 lb	26.5	4.75*	17.25	5.25
Sefina 10	10 oz	15.25	9.25ns	48.5	0.25
Sefina 14	14 oz	15.5	8.25ns	39.5	2
Propriatory	81 g	20.75	19.00ns	31.5	5.75
Steward	11.3 oz	23	6.25ns	44	1.75
Transform	2.25 oz	20.5	10.00ns	23	2.5
Untreated	0	15.25	12.25	26.5	0.5

Results and discussion

Many of the insecticides applied knocked back the abundance of Lygus adults in these plots for a short-period after insecticide application. These trials are conducted in an area that has a substantial amount of forage alfalfa and adults quickly move back into treated plots. The older organophosphate and pyrethroid chemistries and Steward and Transform maintained control of Lygus nymphs both large and small for about a week. At 9 days after treatment control was beginning to break for Lygus nymph control with most chemistries. Aphid control was achieved for a week with applications of Brigade, Mustang, a combination of both and Sefina at 1 week after treatment. In early July we finally had a heat wave and aphid populations naturally crashed in these test plots.

Objective b) Conduct insecticide efficacy tests for candidate Lygus control insecticides during bloom.

On July 19 the insecticides detailed below were applied to 4 replicate plots of 12' by 20' in the equivalent of 20 gallons of water carrier per acre. Plots were sampled by

Table 3. Bloom Insecticides applied on July 19, 2022

1	Untreated Control			
2	Beleaf 50 SG	flonicamid	2.8	oz/acre
3	Proprietary	propriatary	81	g/acre
4	Transform	sulfoxaflor	2.25	oz/acre
5	Steward	Indoxcarb	11.3	Fl oz/acre
6	Sefina	afidopyropen	14	Fl oz/acre

Insecticides were applied on 7/19/2022 to 4 replicate plots of 12' by 20' per treatment by CO_2 powered flat fan boom sprayer. Plots were sampled pre-treatment on 7/18/22 and 2, 6, and 9, days after treatment on 7/21, 7/25, and 7/28, respectively. Sampling was by five 180° sweeps per plot and counting the number of pests including adult Lygus, large and small lygus nymphs, aphids, and alfalfa weevils, and beneficial arthropods including spiders, big-eyed bugs, minute pirate bugs, nabids, lady bird beetles, and lace wing larva capture within the net. Data was entered in spreadsheets. Data was analyzed by analysis of variance and if treatment difference were significant (p<0.01) pairwise t-tests we completed between the untreated control and each respective insecticide treatment. There were no statistical differences in the abundance of beneficials and were usually low among the samples collected and the data is not shown.

Table 4. Pest abundance as adult Lygus and large and small Lygus nymphs per five 180° sweeps with a sweep net.

7/18/2022	Pre-Treatment^{ns}		
Treatment	Adult^{ns}	Large^{ns}	Small^{ns}
Untreated	22.25	3.25	5.00
Beleaf 50 SG	29.25	6.25	14.25
Propriatary	15.75	1.75	18.25
Sefina 14	21.00	2.75	9.00
Steward	26.25	4.25	9.25
Transform	17.50	3.50	8.50

7/21/2022	2 Days after Treatment		
Treatment	Adult$^{0.01}$	Large$^{0.01}$	Small$^{0.01}$
Untreated	38.75	10.00	19.75
Beleaf 50 SG	25.00	5.25**	2.00**
Propriatary	21.25**	6.00**	6.00**
Sefina 14	20.00**	5.75**	3.25**
Steward	30.00	4.25**	4.00**
Transform	12.00**	0.25**	1.75**

7/25/2022	6 Days after Treatment		
Treatment	Adult^{ns}	Large^{ns}	Small^{ns}
Untreated	29.50	7.25	10.25
Beleaf 50 SG	21.50	6.00	6.75
Propriatary	30.00	11.00	4.50
Sefina 14	19.25	5.25	4.50
Steward	25.25	8.50	5.50
Transform	16.50	3.00	3.00

7/28/2022	9 Days after Treatment		
Treatment	Adult$^{0.05}$	Large$^{0.01}$	Small$^{0.01}$
Untreated	31.00	15.75	14.00
Beleaf 50 SG	15.00*	12.00	7.75**
Propriatary	29.75	13.00	10.50
Sefina 14	21.25	8.00**	5.00**
Steward	26.25	14.25	7.75**
Transform	24.50	6.50**	4.00**

Given the results of these field trials alfalfa seed growers have some powerful and effective insecticides available for their use during the bloom period.

Objective c) Conduct Bioassays to quantify resistance status of alfalfa weevil populations to pyrethroid insecticides.

To quantify the dose response of alfalfa weevil populations in Washington State weevil larva were collected at 4 locations in Washington State and transported back to the Environmental and Agricultural Entomology Laboratory at WSU Prosser. These weevils were then subjected to dose response bioassay via our Potter precision spray tower. Larva populations were collected from sites including an alfalfa forage field in Goldendale, an alfalfa field at WSU Othello, an alfalfa field near Gardena, and an alfalfa field at WSU IAREC. The 4 insecticides tested included the registered products Warrior II (lambda-cyhalothrin), Mustang Maxx (zeta-cypermethrin), Baythroid (beta-cyfluthrin) and Lorsban Advanced (chlorpyrifos). Serial dilutions were completed for each insecticide in a dilution equivalent to 20 gallons per acre at 100%, 75%, 50%, 25%, 10%, 5%, and 0% of the maximum field rate for Lorsban Advanced and Warrior II 75%, 50%, 25%, 10%, 5%, and 0% of the maximum field rate for Mustang Maxx and Baythroid. Each treatment was applied to 4 replicates of 5 weevil grubs in a Petri dish with a filter paper bottom in 2 ml of solution in our Potter precision spray tower. The weevil larva were evaluated at 24 and 48 hr after treatment for mortality and survivorship. Subsequently our data evaluations were completed on the weevil morality after 48 hr of exposure due to greater consistency of results. Weevil larva were considered dead when they failed to respond to being touched with a fine camel hair brush.

Figure 1. Dose response of alfalfa weevil populations in percent mortality ± Std error to chlorpyrifos (Lorsban Advanced)at concentrations equivalent to 0, 5, 10, 25, 50, 75, and 100% of the maximum field rate in parts per million (mg/liter)

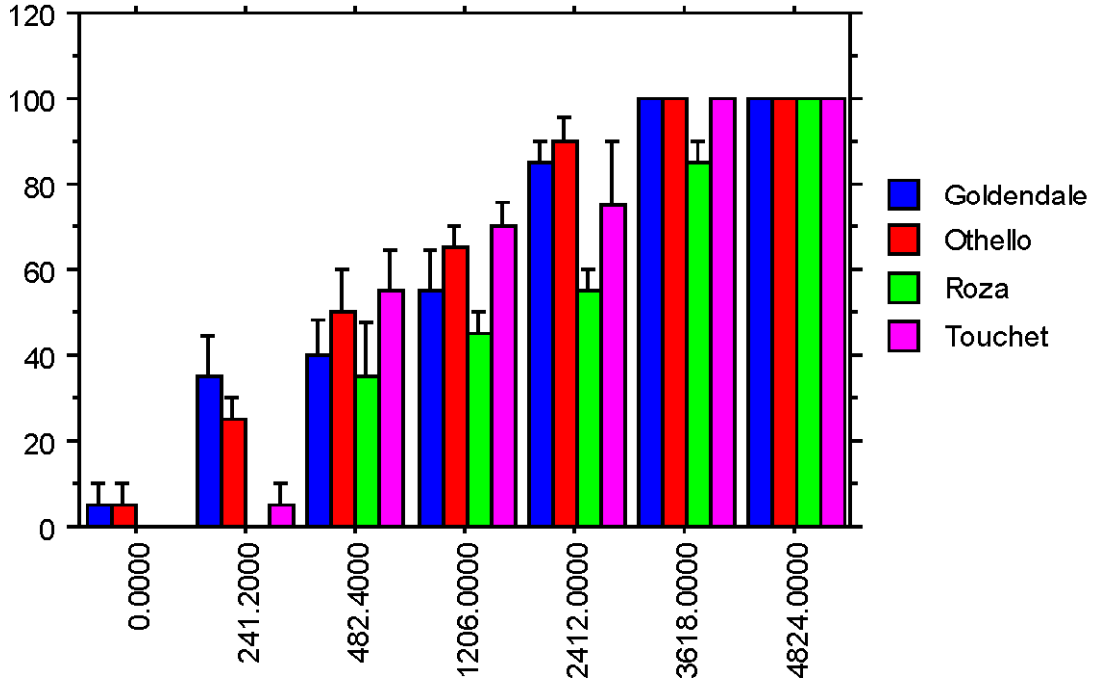


Figure 2. Dose response of alfalfa weevil populations in percent mortality ± Std error to lambda-cyhalothrin (Warrior II) at concentrations equivalent to 0, 5, 10, 25, 50, 75, and 100% of the maximum field rate in parts per million (mg/liter)

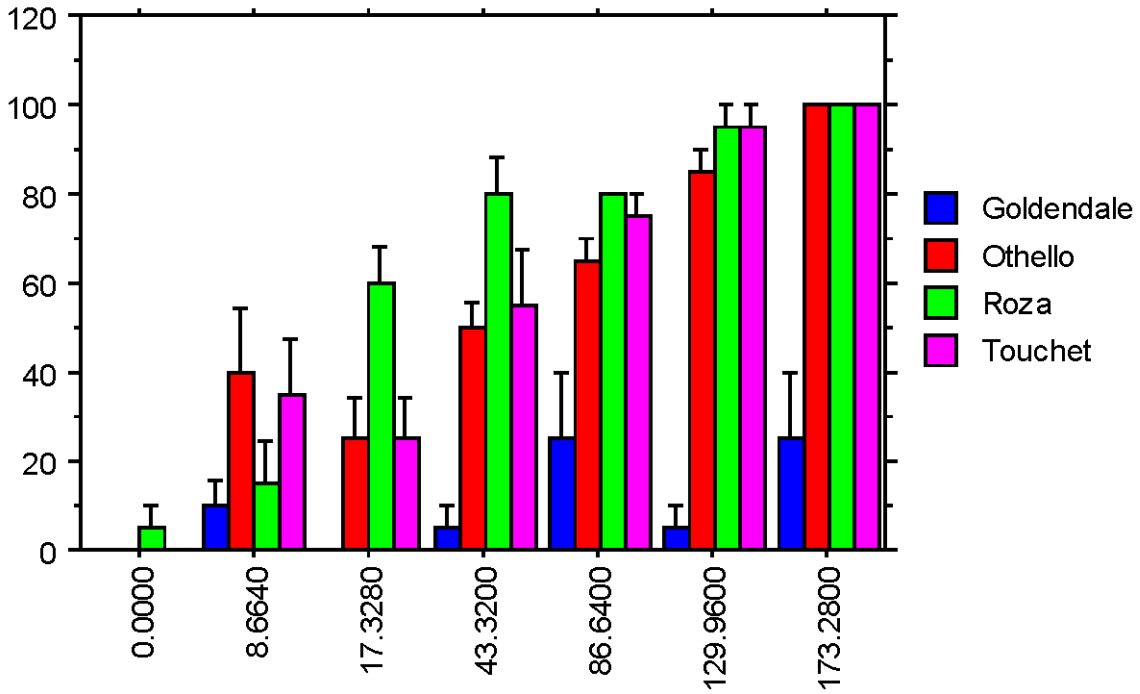


Figure 3. Dose response of alfalfa weevil populations in percent mortality ± Std error to zeta-cypermethrin (Mustang Maxx) at concentrations equivalent to 0, 5, 10, 25, 50, and 75% of the maximum field rate in parts per million (mg/liter)

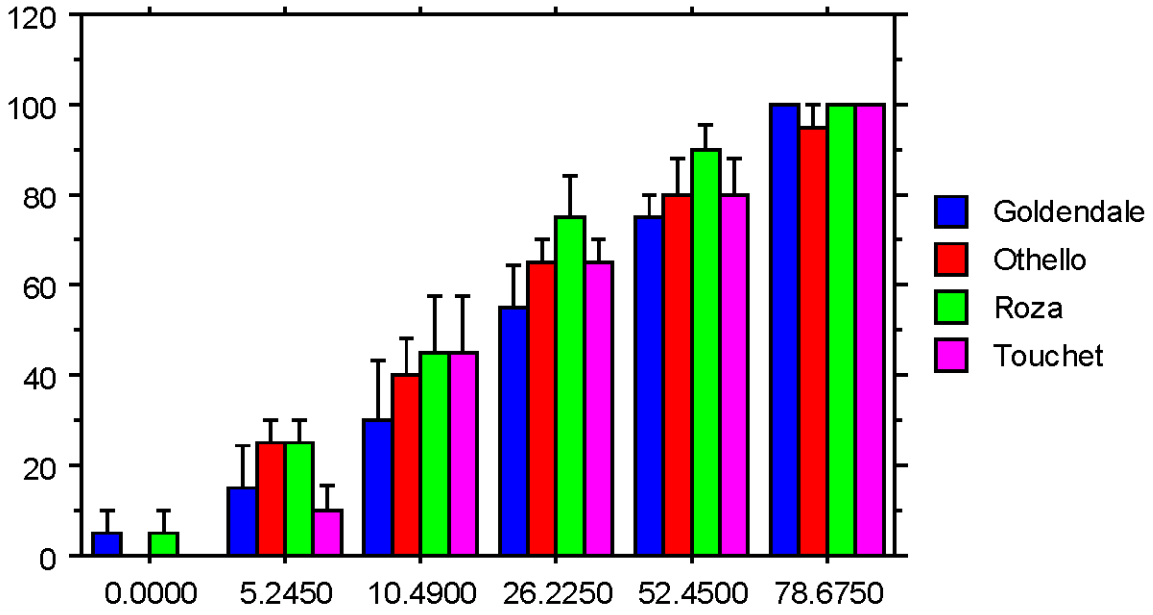
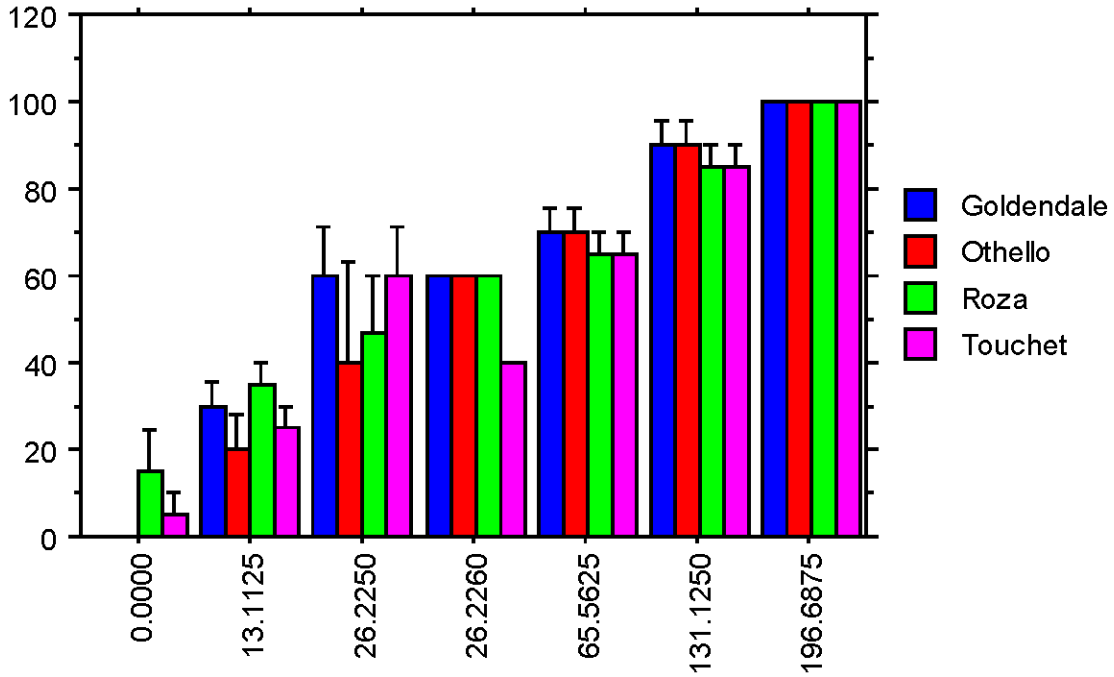


Figure 4. Dose response of alfalfa weevil populations in percent mortality ± Std error to beta-cyfluthrin (Baythroid) at concentrations equivalent to 0, 5, 10, 25, 50, and 75% of the maximum field rate in parts per million (mg/liter)



Among all the 4 populations tested against the organophosphate chlorpyrifos and the 3 synthetic pyrethroids, only the Goldendale population exhibited resistance to lambda-cyhalothrin and the Roza population exhibited resistance to chlorpyrifos. The revocation of the tolerance on using Lorsban Advanced (chlorpyrifos) for alfalfa weevil control will only put increased pressure on the synthetic pyrethroids and increase the likelihood of resistance developing to the pyrethroids.

BEE FRIENDLY FARMING® HANDBOOK

POLLINATOR PARTNERSHIP

Bee Friendly Farming® has three distinct categories of participation – **BFF CERTIFIED, BFF PARTNER and BFF GARDEN**. Each has different requirements and benefits. The following pages detail each of the three categories. For any questions contact Miles Dakin at 707-321-7165 or Miles@pollinator.org.

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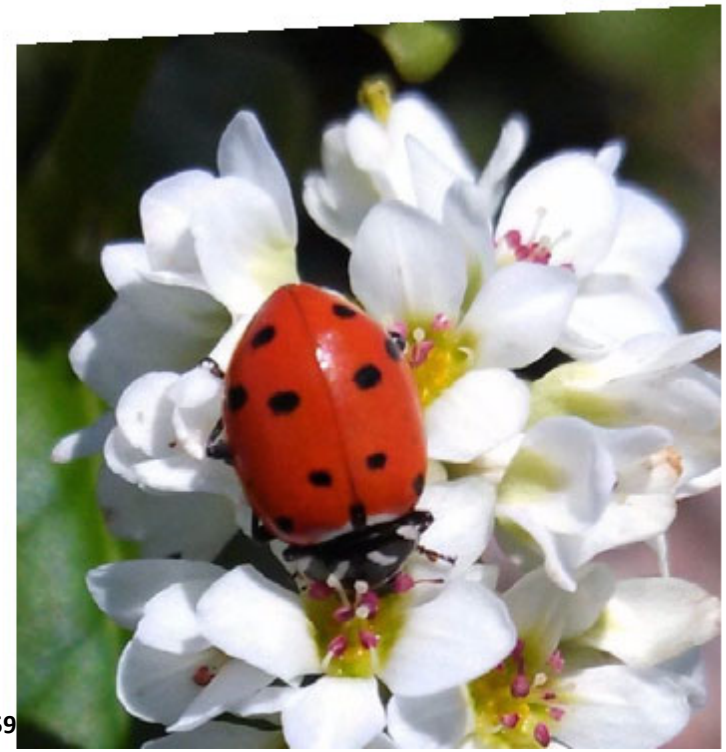
BEE FRIENDLY FARMING®

Purpose

Bee Friendly Farming® (BFF) provides guidelines for farmers and growers to promote pollinator health on their lands. **BFF** strives to set standards for sustainable farming on important concepts like planting pollinator food resources, providing nesting habitat, and incorporating an integrated pest management strategy. Through **BFF,** farmers, home gardeners, and private or corporate sponsors can be directly involved in providing resources necessary to support pollinators. By providing a low-cost membership program, growers and gardeners can spend valuable time and money getting plants and seeds in the ground to support pollinators.




Lynmar Estates

History

Bee Friendly Farming® was acquired by Pollinator Partnership in 2013 at the fifth anniversary of its existence. **BFF** was founded by Kathy Kellison in 2008 through the Partners for Sustainable Pollination (PFSP) in order to work with growers to promote and provide pollinator habitat in agricultural landscapes, in collaboration with Sam Droege, Mace Vaughan, Dennis vanEnglesdorp, Marla Spivak, Randy Oliver, Robbin Thorp, Karen Strickler, Gerry Miller, Jeff Anderson, Phil Giles, and Gene Brandi, among others. Pollinator Partnership has continued to expand and grow **BFF** into the program it is today. A Taskforce of the North American Pollinator Protection Campaign (NAPPC), which includes scientists and farmers, meets monthly to oversee the program.

P2 is dedicated to further expanding and developing this program and supporting members in providing habitat for pollinators. In the beginning, the goal of **BFF** was to engage as much landscape as possible and manage it responsibly to welcome and sustain bees and other pollinators. It was used to raise awareness in the consumer of the importance of patronizing responsible producers. This basic goal has not changed much in the long history of **BFF,** but it certainly has been refined, improved and expanded to embrace a variety of approaches to support pollinators responsibly.

BEE FRIENDLY FARMING® CATEGORIES TODAY

Bee Friendly Farming® Categories Today

The Bee Friendly Farming® program has expanded into three distinct categories - **CERTIFIED, GARDEN,** and **PARTNER.** These three categories provide avenues for participation for a variety of landscapes and goals. Each category has its own requirements and benefits. **BFF CERTIFIED** is most appropriate for farming operations. It is rigorous and requires the most stringent compliance while allowing the use of the **BFF** logo to those whose diligence is authenticated and rewarded.

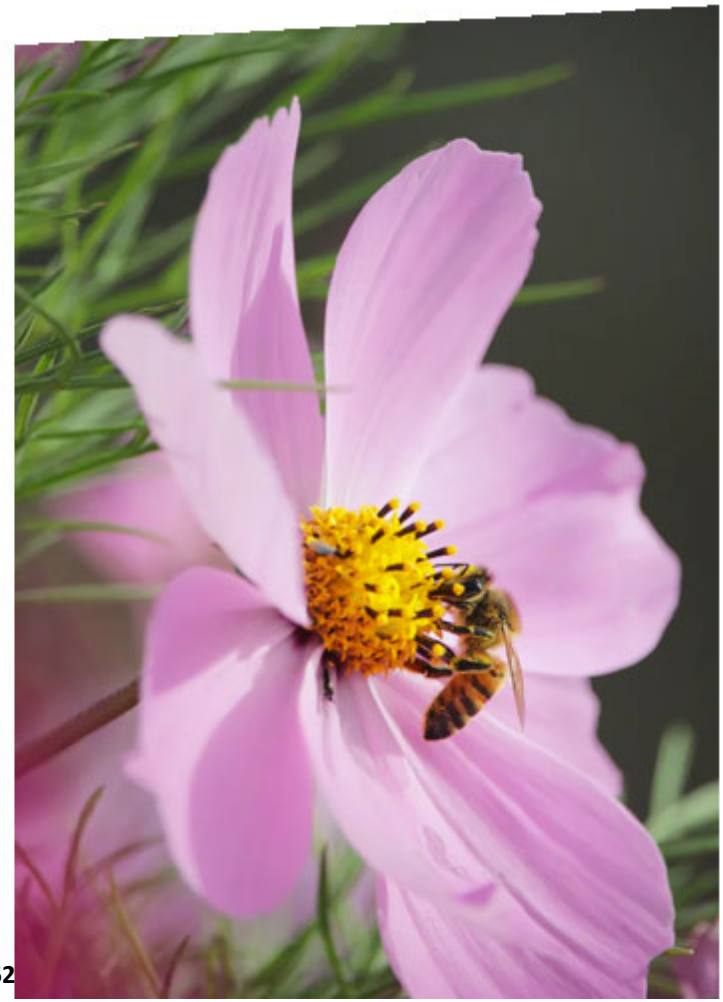
BFF GARDEN is designed for home and community gardens that maintain pollinator habitat and management practices. Both of these categories recognize habitat on the land and the management practices that keep habitat safe and supportive for pollinators.

BFF PARTNER is a designation for members of the community, corporate or individual, who do not necessarily maintain pollinator habitat but wish to support the program. Their financial backing allows for more habitat for pollinators and more support for growers and partners.

Apiarists

BFF is grateful for and supportive of our commercial apiarists community members and encourages them to apply for a category that matches their needs and goals. For apiarists that do not manage their own lands, the PARTNER category has the addition of a specific apiarist category that will provide them with resources and support specific to their needs.

The designation of BFF categories allows P2 and participating members to connect and interact in distinct ways unique to their capacity and goals. This means the ability to design specific outreach and participation materials for each category.



How to determine the right BFF category

In order to choose the right category for each member, it is important to recognize the main goals of each. **CERTIFIED** is designed for large scale commercial farms, ranches, wineries or other land-use businesses that grow food or other resources. This designation gives the land, and product grown on it, the ability to be labelled with the **BFF** logo on marketing and packaging material. **PARTNER** is designed for members who want to provide more financial support to the program and gives logo use on promotional materials. For a small, non-commercial garden, **GARDEN** would be the ideal designation for them. This level does not receive logo use rights as it is designed for non-commercial lands.

BFF Application Fees

Members may apply to one of the three **BFF** categories through the online application. Along with the information required to meet the criteria for the chosen category, a non-refundable application fee is required for **BFF GARDEN** and **CERTIFIED**. The application fee for **GARDEN** is $20. The fee for **CERTIFIED** is $45. Upon acceptance into the program, this application fee will count as the first year membership fee, upon which an annual recurring fee of an equal amount will be required. **BFF** strives to provide resources and engagement with members at a minimal cost. By keeping membership fees low, **BFF** hopes that members can further enhance the environment to support pollinators. Members can use the money that might have gone to more expensive certification programs to plant more foraging habitat, implement new management strategies, or spread the word about pollinator health!

Checking in and getting checked out: compliance with the rules

Along with the qualification criteria for each category and maintaining annual membership fees, each category has compliance criteria. **CERTIFIED** members must file a compliance form once every 3 years. These forms will be audited by the **NAPPC BFF** Taskforce. Field visits will take place with between 3 and 6% of the **CERTIFIED** memberships. **PARTNER** compliance will require a short annual survey to determine how the **BFF** logo is being used. **GARDEN** compliance will be maintained annually through a short survey.

Through these compliance methods, P2 can access habitat management practices, the success of habitat installations, benefits provided, and any additional changes that the land-owners have made to further support pollinators. Verification of compliance will take place through review of each submission by the **BFF** administrative and coordinator team, as well as through the North American Pollinator Protection Campaign **(NAPPC) BFF** Task Force. Just like the federal government (which gives millions of dollars to farmers) **BFF** does not have third-party verification. First, we trust that farmers are trying to do their best and it's in their best interest to do the right thing for their farm and future; in addition, they are asked to report extensively in the required compliance form checked by the **NAPPC BFF** Taskforce and the **BFF** staff. Second, rather than expensive third party policing, **BFF** invests in more seeds on the ground – something that will make a real and lasting difference. And third, since **BFF** has designated staff and advisory board members specifically tasked with **BFF** membership and compliance, **BFF** builds direct relationships with growers and supports both their productivity and the sustainability of pollinators. **BFF** combines the best of science and support for farmers and pollinators.

BEE FRIENDLY FARMING® CERTIFIED

The **BFF** flagship certification has been revamped and redesigned to highlight and support farms, wineries, ranches, and other commercial land-use operations that promote and support pollinator health. By becoming **Bee Friendly Farming® Certified** the grower helps preserve and protect pollinator populations by implementing positive, incremental, substantiated changes on agricultural landscapes.

Each **Bee Friendly Farming® Certified** farm is an essential part of keeping pollinators healthy and the food supply abundant, and receives use of our **Bee Friendly Farming®** logo and can be featured on the P2 blog for product promotion.

Criteria for Certification:

1. **Offer forage providing good nutrition for bees on at least 3% of land. Forage includes cover crops, if they are left to bloom.** Pollinators require year round food and habitat other than crops. By planting flowering plants that produce nectar and pollen, we can support our pollinators year round.

Various pollinator friendly perennial plants

2. **Provide bloom of different flowering plants throughout the growing season, especially in early spring and late autumn. There is no minimum land coverage for seasonal bloom.** During the growing season, it is important to provide pollinators with alternate food sources that offer a complete diet. Both commercial honey bees and native pollinators benefit when provided with diverse food sources.

Cover crop in almonds

3. **Offer clean water for bees, if not inhibited by government-mandated water restrictions.** In large scale commercial farming operations, it is important to provide clean water for pollinators. Irrigation canals, holding ponds, lakes, or natural bodies of water can count for certification.

4. **Provide permanent habitat for nesting through features such as hedgerows, natural brush, buffer strips, or bare ground.**

Bee Friendly Farming® winery with various flowering plants

5. **Pest Management (IPM); reduce or eliminate the use of chemicals.** Many insecticides negatively impact pollinators and have led to a dramatic decline in insect populations over the last century. With this in mind, it is important to develop IPM programs that take into consideration the farmer's needs and pollinator health. BFF Certified farmers are expected to practice IPM and reduce or eliminate the use of chemicals.

6. **Pay the annual certification fee. In order to support the BFF program, there is an annual certification fee.** This fee accompanies logo-use. Larger growers are encouraged to contribute an additional donation with increasing acreage.

7. **Complete the compliance form once every 3 years (audited by the BFF Taskforce).** A compliance form must be submitted every 3 years and will be audited by the **BFF** Taskforce. The project coordinator will conduct field visits on 6% of compliance submissions annually.

Demonstration area of BFFC with variety of plants

Benefits of Bee Friendly Farming® CERTIFIED

- Use of **BFFC** logo on products
- Sustainable sourcing and consumer demand for **BFF CERTIFIED** products
- Access to **BFF** team for support, including plant suggestions, connection to sourcing, site visits etc. These recommendations are built on the best science for both pollinators and your crop
- Access to seed grants (e.g. Flow Hives grant, Seeds for Bees)
- Connections to corporate sponsors/ partners
- Membership to a network of like-minded growers
- Access to branded hats, signs, etc. on website store
- Monthly newsletter, blog, website, other features to learn from and be featured in
- Feedback survey
- Complete privacy policy for data usage; farmers can feel comfortable sharing information with **BFFC**.

Checklist for Application to become Bee Friendly Farming® CERTIFIED

It should take less than 45 minutes to fill out and submit online if you use the checklist below to prepare the information you will need:

- ☑ Proof of current good standing in other accepted certifications (CASP, Live Certified, Bee Better Certified, USDA Certified Organic, among others). Uploading these files will allow you to bypass significant portions of the form. If your certification is not an available option in the application, please reach out to isaac@pollinator.org for more information or to have it considered.
- ☑ A list of all known plant species on your farm that provide forage for bees and other pollinators
- ☑ Images of your forage habitat
- ☑ Images of your clean water sources
- ☑ Images of potential bee nesting habitat
- ☑ Farm map file(s) (PNG, PDF, shapefiles, KML/KMZ, etc.) that shows a clearly defined property line of the area being certified and includes boundaries and labels for each ecological infrastructure (temporary floral resources, permanent floral resources nesting habitat, water sources). Include acre counts for each delineated area.
- ☑ Pest management protocol, including monitoring/ identification practices, decision making steps, prevention techniques, intervention (application conditions, drift management, etc.), evaluation, and resistance management.
- ☑ Create a PayPal account if you do not already have one. (Please reach out to isaac@pollinator.org if you need to use another payment method.)

You are ready to fill out your certification application. Be sure to allow the form access to your location when prompted, and enter your farm's primary phone number to make future compliance forms much easier; this information is how you will retrieve your application data.

Integrated Pest Management

One of our goals at Pollinator Partnership is to provide science-based resources and expertise that make incremental changes to land-use practices. The Bee Friendly Farming program highlights and supports farming operations that set the standard for pollinator health management. The program is built on four criteria that support all pollinators: providing diverse forage for pollinators, providing and managing nesting habitat, providing clean drinking water, and developing mindful pest management programs. One of the keystones of the program is working with farmers to develop Integrated Pest Management (IPM) plans that consider pollinator health, while meeting the goals of the farmer.

The Bee Friendly Farming® Certified application focuses on the main principles of IPM:

- Pest monitoring and identification
- Decision making based on monitoring and thresholds
- A multi-faceted approach that combines chemical, physical, biological, and cultural control methods
- Prevention of infestations
- Evaluation and improvement of management strategies
- Resistance management

While each of these fundamental aspects of IPM play an important role in optimizing management of pests, careful consideration of pollinator health should be taken in each of these steps to support pollinators without limiting efficacy of pest management strategies.

The **BFF Certified** application requires adoption of IPM. Information on each of the IPM principles.

1. Pest monitoring and identification: Proper identification and monitoring of pests is vital in understanding the specific situation and potential mitigation with any possible pest infestations. This series of questions asks for detailed information about how monitoring occurs, by whom, where the information for identification is coming from (extension guidelines, etc.), and if records are stored. This informs management decisions that might affect pollinators.

2. Decision making based on monitoring and thresholds: Management decisions should be based on monitoring and assessing if threshold levels are met. Models are commonly used to help make decisions about the timing of management practices. These can be based on initial trap catches and weather data. By using these types of models, growers can make science-based decisions in developing management plans. This is important because it ensures that growers are applying management strategies at the proper time and avoiding any unnecessary applications, reducing pesticide exposure to pollinators.


Antony John

3. A multi-faceted approach that combines chemical, physical, biological, and cultural control methods: IPM benefits from a combination of management approaches that can use different modes of action and strategies, taking advantage of physiological, ecological, and behavioral characteristics of the target pests. These non-pesticide approaches reduce potentially toxic exposure to pollinators. The means of applying chemicals are also important in mitigating exposure to pollinators. Growers are required to use a multi-faceted approach that combines physical, biological, chemical, and cultural control methods and are required to demonstrate which management strategies they are implementing.

4. Prevention of infestations: An important aspect of IPM is the principle of avoiding potential infestations. Small steps can be taken to mitigate outbreaks, many of which directly benefit pollinators. Growers are required to practice at least 2 preventative measures.

5. Evaluation and improvement of management strategies: Many of these principles can and may need to be adjusted as seasons change. Adapting farming practices to new methods, changes in the environment, or emerging pests are essential to developing impactful IPM programs. We are interested to learn more about the internal process and decision making for adapting to these situations and how pollinator health is incorporated in these decisions.

6. Resistance Management: Pest populations can develop resistance to specific pesticides through continued use of the same Mode of Action (MoA). Alternating MoAs, applying at appropriate rates and timings, calibrating equipment, and many other techniques can all help prevent resistance evolution. A passing BFF application will demonstrate the use of at least one resistance management technique recommended by the Insecticide Resistance Action Committee (IRAC).

At **Bee Friendly Farming®**, we understand how complex and important IPM is to our members. We believe that these key principles provide the growers a framework to not only protect their livelihoods, but incorporate pollinator health and awareness. We also understand that practices and situations change from year to year, and we continue to listen and adapt to the needs of our members and pollinators.

BEE FRIENDLY FARMING® PARTNER

In order to support our pollinators, commercial and individual sponsors can become a **Bee Friendly Farming® PARTNER. BFF PARTNER** registers sponsorship from companies and individuals, but also includes commercial apiarists who do not actively manage their own landscapes, but wish to support the program. These sponsorships provide the resources necessary to build and maintain this program, benefiting pollinator health and food security. Sponsorships and donations will not only benefit pollinators, they will benefit us all. Bees and other pollinators are threatened, but with support from **Bee Friendly Farming® PARTNER** members, P2 is developing programs that help improve pollinator health on landscapes across the globe.

FFD Vineyard

DONATION levels and BENEFITS of Bee Friendly Farming® PARTNER

Honey $45

- Restricted to Apiarists
- Use of **BFF** logo on website, promotional material
- Monthly **BFF** communication
- Listed on **BFF** website

Pollinator Friend $500

- Use of **BFF** logo on sponsor website
- Listed on **BFF** website with link
- Monthly **BFF** communication

Copper $1000

- Same as Pollinator Friend PLUS
- Sponsor logo on **BFF** Website

Bronze $5000

- Same as Copper PLUS
- Listed in **BFF** Communications
- Listed in **BFF** webinar credits

Silver $10,000

- Same as Bronze PLUS
- Listed in Ag Ad
- Plant Consultation

Gold $25,000

- Same as SIlver PLUS
- Link with product shot
- Logo on Annual poster
- Ability to print logo on packaging
- Plant consultation with planting supervision
- Large sign

Platinum $50,000

- Same as Gold PLUS
- Large sign – personalized
- Your operation/product featured in webinar

Engagement:
- Interviews, blogs, feature on website
- Monthly Newsletter

Compliance:
- Use of logo MUST include either “Partner of **BFF**” or “**BFF Partner**”
- Annual survey to determine logo use
- Annual donation

BEE FRIENDLY FARMING® GARDEN

BFF's newest and most unique category of membership is specifically designed for home and community gardeners who promote and provide habitat and pollinator health in non-commercial settings. **Bee Friendly Farming® GARDEN** has been designed to distinguish these members from commercial farming enterprises or sponsors, as well as highlighting gardeners who follow the specified guidelines in providing for pollinators and offering a diverse and permanent habitat source at a scale at or above a small home garden.

In addition, **BFF GARDEN** registration means limiting or avoiding the use of harmful pesticides on blooming plants, and paying the annual $20 registration fee.

BFF GARDEN membership does not include logo use except in special circumstances. Contact the **BFF** team for use information. Habitat signs and support are available, as well as the opportunity to be featured on the P2 blog.


Gail Vandersteen

John Monarch

Criteria for Bee Friendly Farming® GARDEN

- Plant native plants to provide nectar and pollen throughout the bloom season.
- Provide nesting sites through permanent plantings, bare ground, tree stumps, or by building bee boxes.
- Reduce or eliminate the use of pesticides. Use Integrated Pest Management (IPM). Apply no chemicals to blooming plants or when pollinators are foraging. (See here for more information).
- Pay the annual membership fee.
- Complete annual garden surveys.

Benefits of Bee Friendly Farming® GARDEN

- Support for and inclusion in the **BFF** program.
- Access to hats, signs, etc. on website store
- Blog/ social media feature - My Pollinators - My Story
- Monthly email newsletter
- Access to gardener-specific information
- Provide feedback through annual surveys

BEE FRIENDLY FARMING® LOGO USE

As outlined in each category section, logo use is restricted to **BFF CERTIFIED** and **PARTNER** members. **BFF GARDEN** designation DOES NOT include logo use outside of the ability to post official **BFF GARDEN** signs in designated habitat. **BFF GARDENS** may not use the **BFF** Logo for any monetary gains. Prior to application submission, all applicants must agree to the **BFF** Logo Use Agreement and follow all criteria and requirements listed in the agreement. If a member violates the agreement, their membership may be revoked, unless a resolution is made between P2 and the member. **CERTIFIED** and **PARTNER** members shall use their respective logos in a manner similar to below:

CERTIFIED

PARTNER

It is possible that members of these two categories can modify the Bee Friendly Farming® logo to not include their category designation, but must specifically state their designation in writing above or below the logo use. This modification is laid out in the **BFF** Logo Use Agreement. As an example:

"This land is a **CERTIFIED Bee Friendly Farm**® by Pollinator Partnership"

"We are a proud **PARTNER of Bee Friendly Farming**®"

POLLINATOR PARTNERSHIP

bpgrower.com

BEAVER PLASTICS

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What bees want... what producers need

- Patented raised orientation
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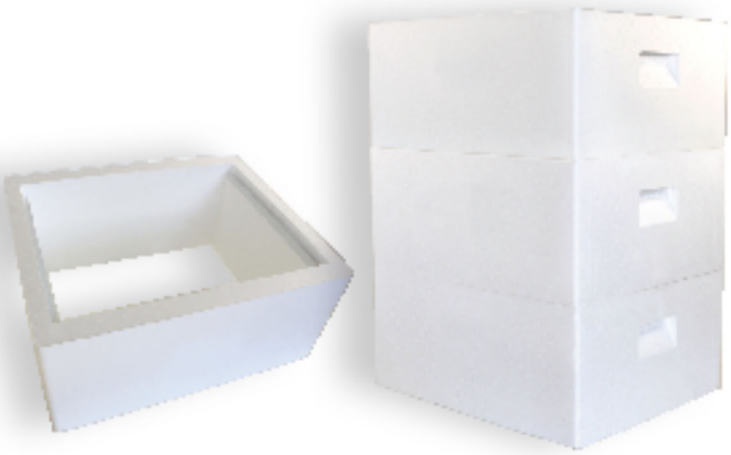

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