ENREC Apiaries (4 hives/site)

- 6 (2019-2020)
- 3 (2021-2022)

  -Insect Building

  -Agronomy

  -Fireshop

Off-Site Apiaries (2 hives/site)

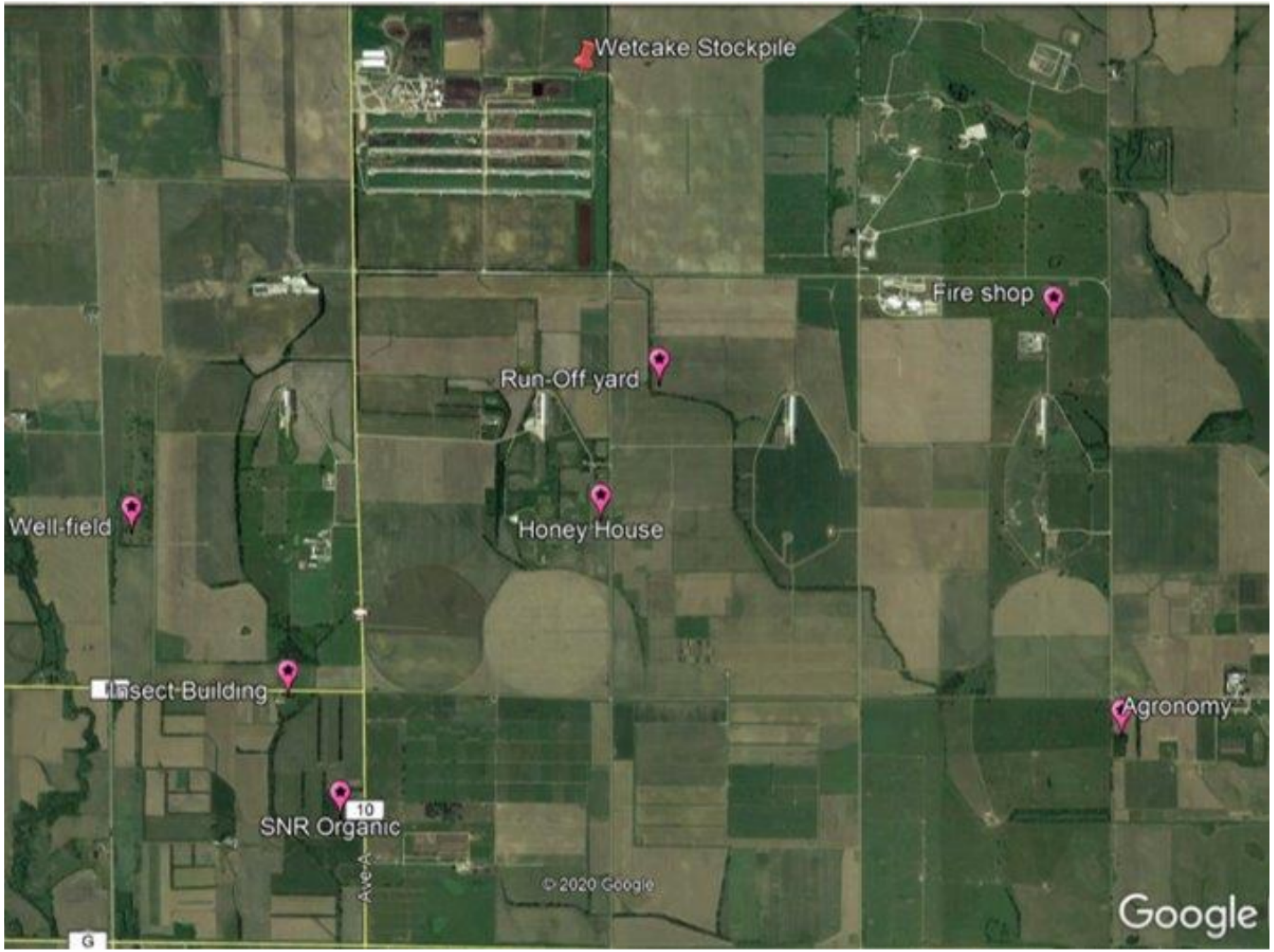
- 4 private farms (2021)

  -JW (West)

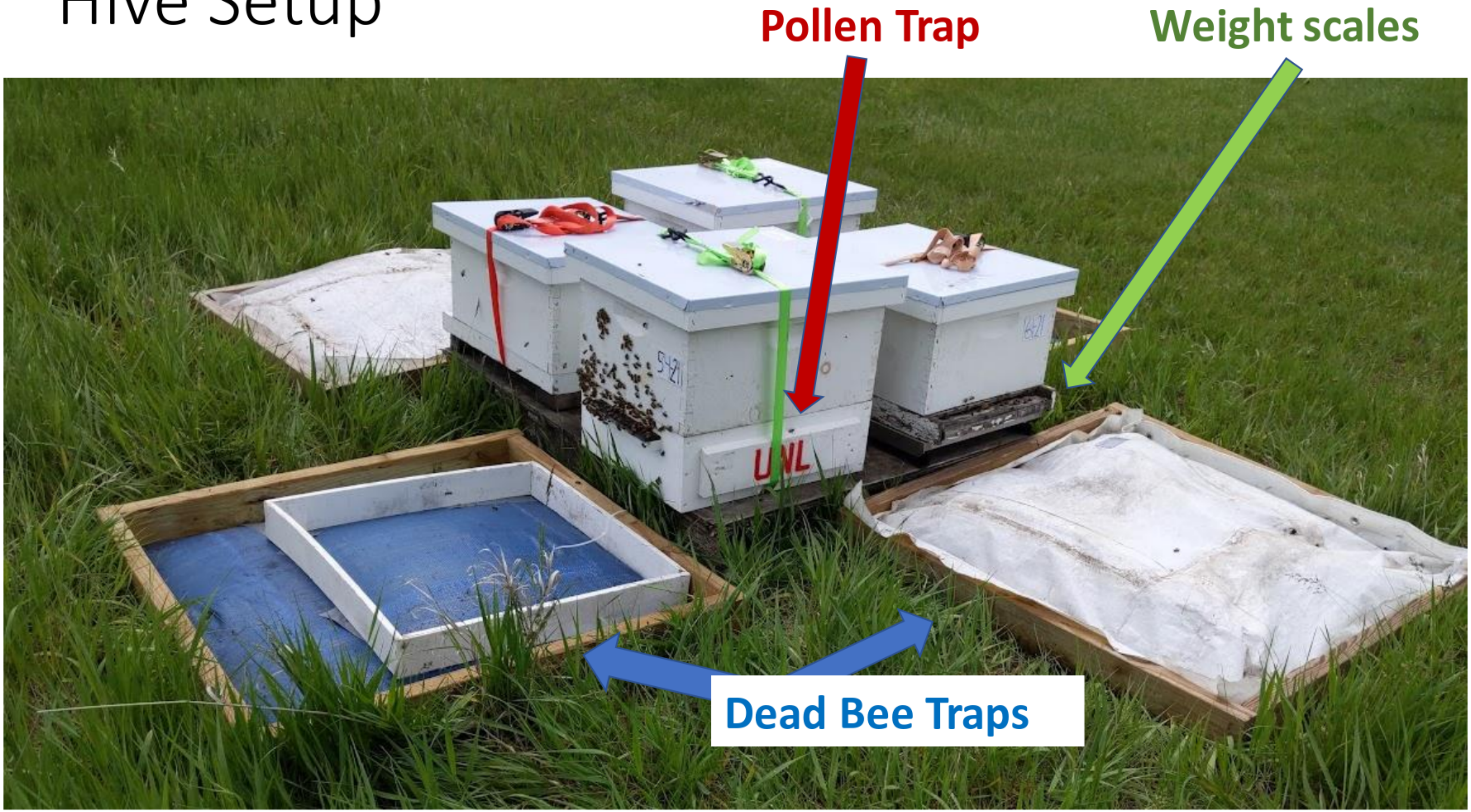
  -PD (North)

  -CM (East)

  -SK (SE) late 2021 start
- 4 same farms (2022)



Hive Setup



New hives started each year

Sister colonies equal in population

Each with a healthy laying queen

Allowed to build-up in Lincoln during early Spring

Setup with new & old equipment

Randomly placed in locations

In-hive samples taken from new comb


adidas

Sample Processing for Pesticide Testing

In-hive pollen samples (from each hive in late summer)

-2020-tested

-2021 Samples -> ready to send to USGS

In-hive nectar

-2020-tested

-2021 Samples -> ready to send to USGS

Trapped pollen (biweekly) (May-Sept 2020-21):

-2020 Samples -> DNA Profile identify species

-2021 Samples -> prepared and ready to send to USGS

Vegetation (flowers, leaves)

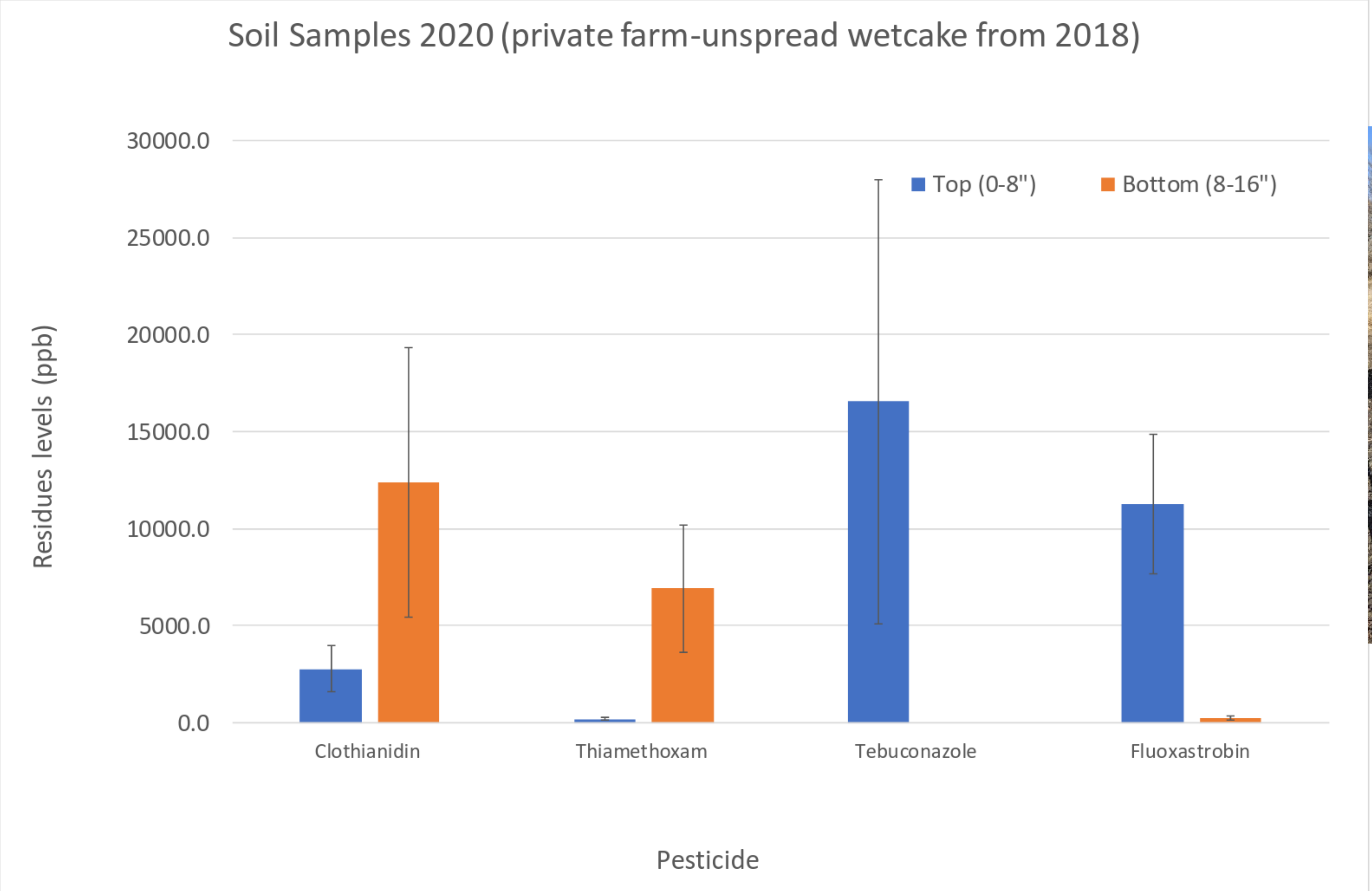
-2018, 2019 –archived samples

-2020- tested

-2021 -processing

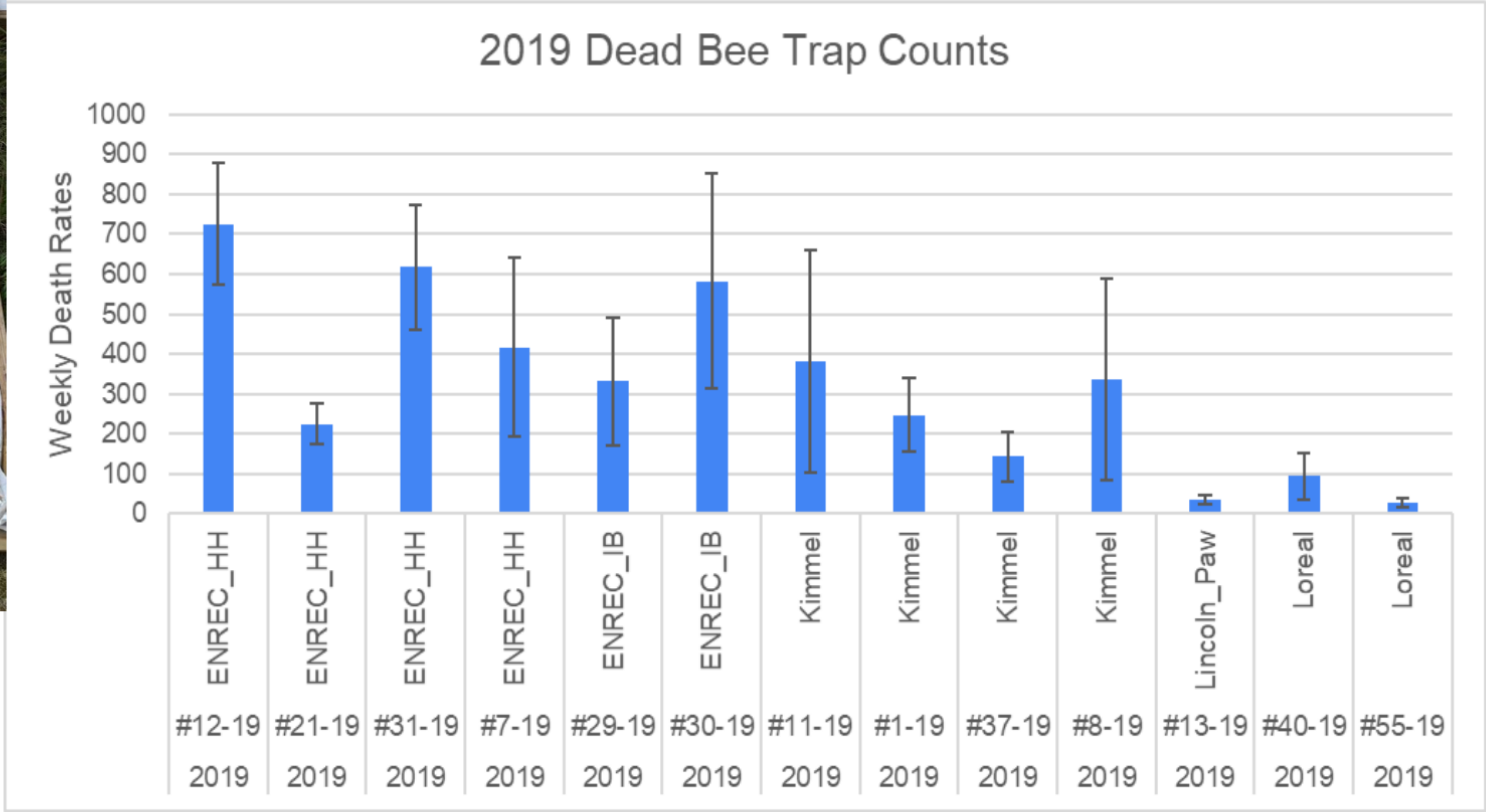
Clothiandin
(ug/L or ppb)

2020 Samples	**high**	**average**	**n**
Wildflowers	42.8	13.3	6
In-Hive Pollen	284.5	109.2	10
In-Hive Nectar	1.8	0.2	8

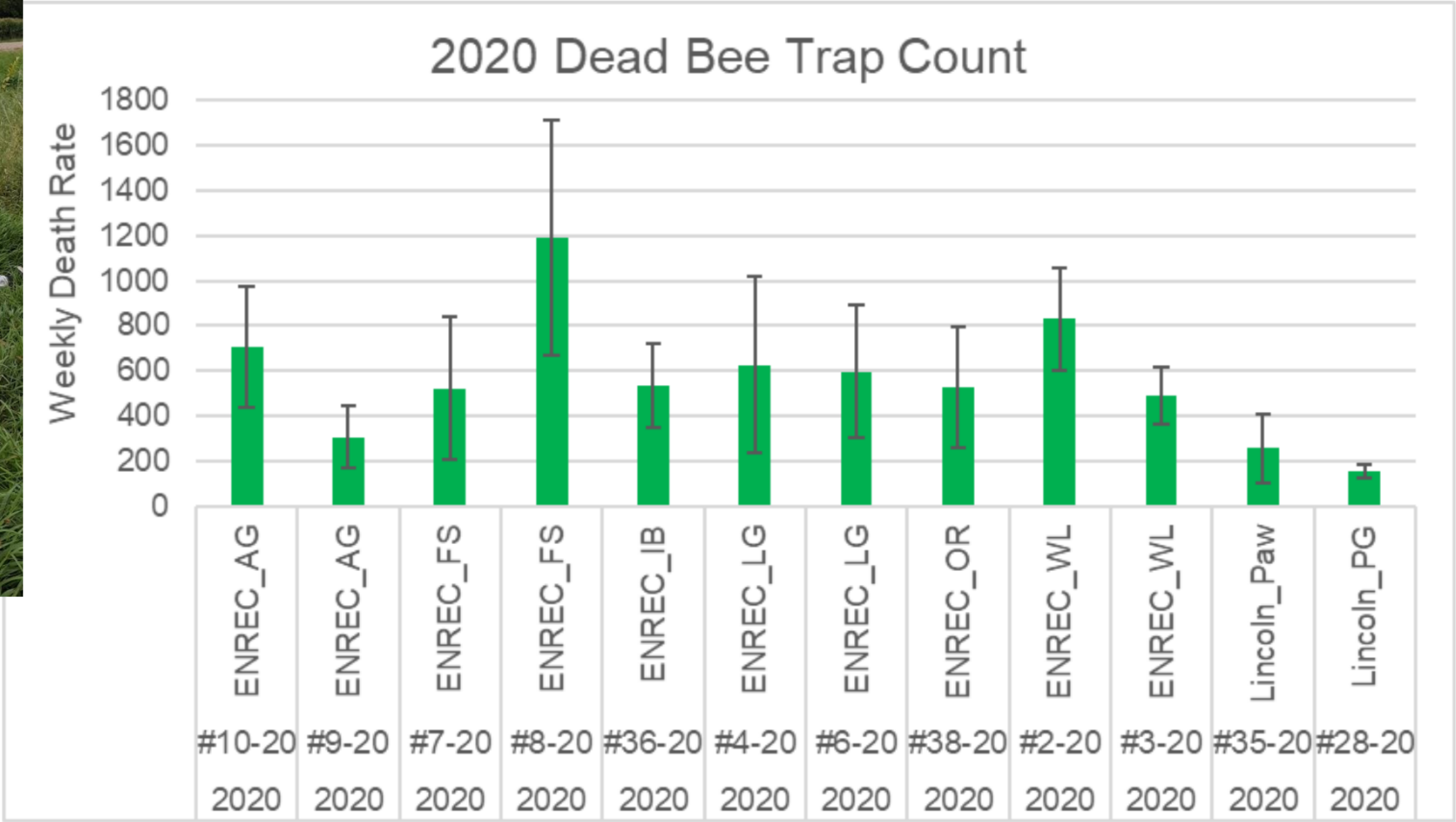


Additional soil and sticky trap dust samples 2018, 2019, 2020 sent to WSL

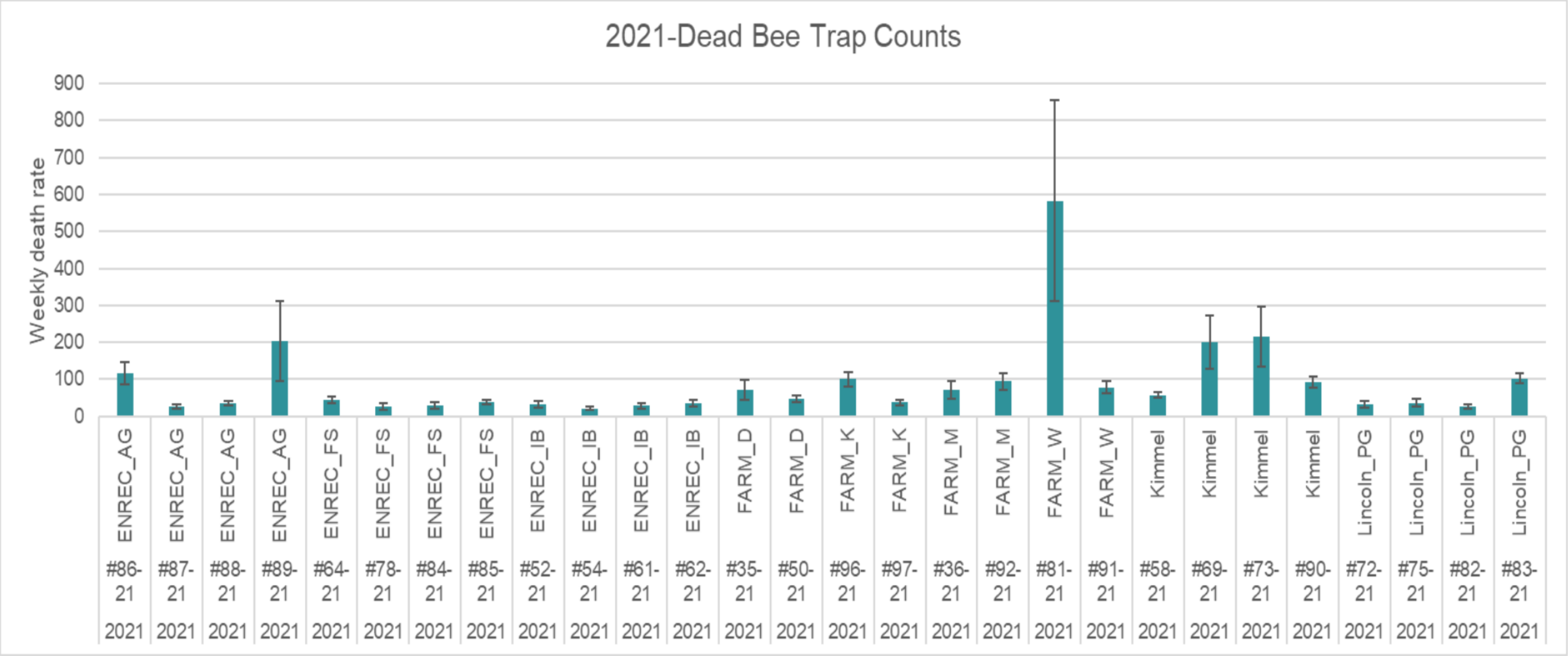
Preliminary data



Preliminary data



Preliminary data

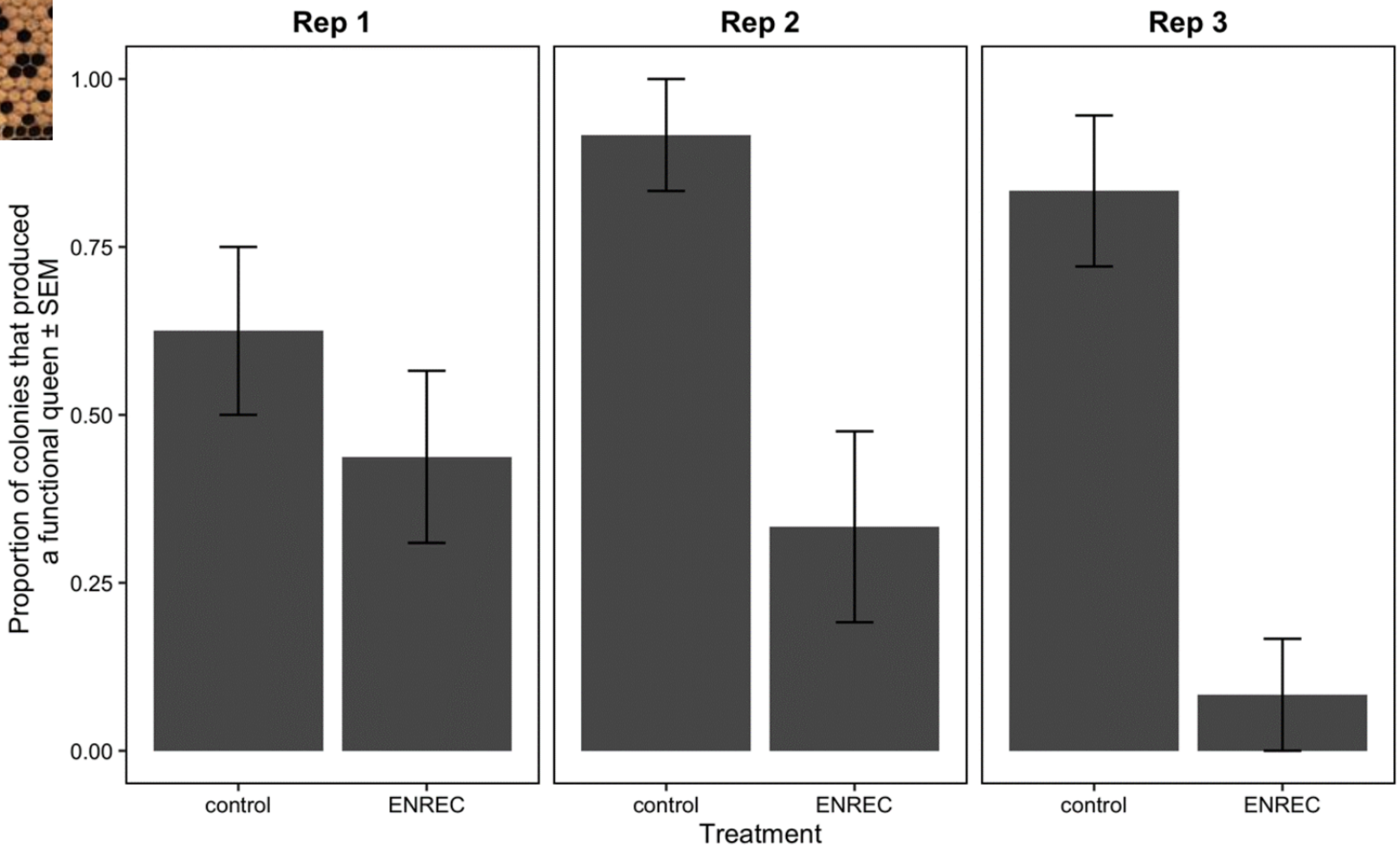


COLONY SURVIVAL (# alive/total)

	WL	OR	HH	AG	FS	IB	Farm W (West)	Farm D (North)	Farm M (East)	Farm K (SouthE)	Lincoln	NE City
2019	**0/4**	**0/4**	**0/4**	**0/4**	**0/4**	**0/4**	**-**	**-**	**-**	**-**	**4/4**	**4/4**
2020	**-**	**-**	**-**	**0/4**	**0/4**	**0/4**	**-**	**-**	**-**	**-**	**4/4**	**3/4**
2021	**-**	**-**	**0/2**	**1/4**	**1/4**	**1/4**	**0/2**	**1/2**	**1/3**	**2/2**	**3/4**	**2/4**
2022	**-**	**-**	**-**	**2 old 4 new**	**2 old 4 new**	**2 old 4 new**	**2 new**	**2 old 2 new**	**2 old 2 new**	**2 old 2 new**	**4 new**	**4 new**

Weekly dead bee trap counts continuing
Colony health measures (Adult & brood populations, mite loads)

Queen rearing



Behaviors monitored

- Brood care
- Queen attendant
- Comb building
- Grooming
- Ventilation
- Cell cleaning

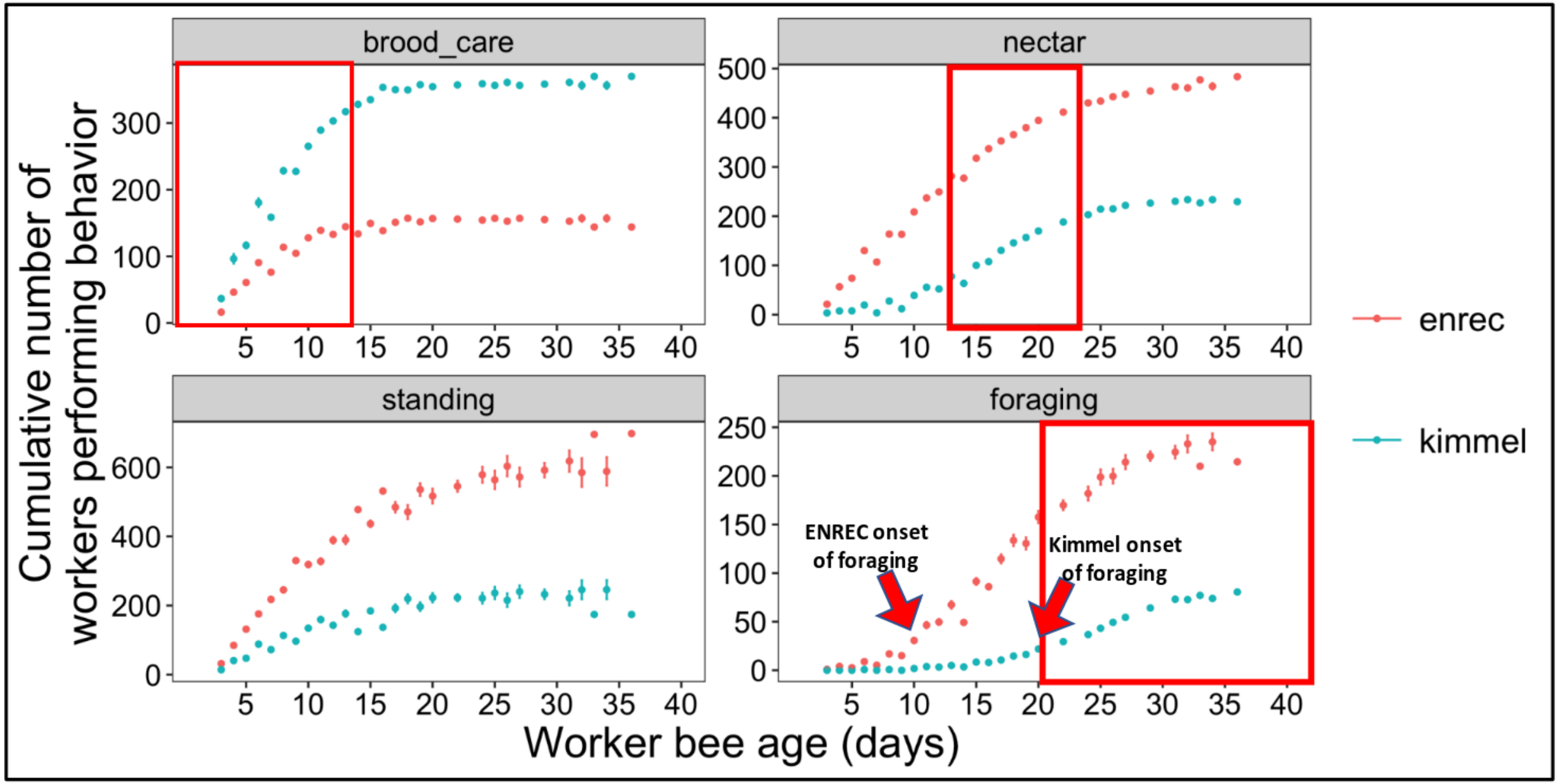
(Collison 2016)

(Photo by Kathy Keatley Garvey)

Hive functions & behaviors

Project *Apis m.*

Preliminary results



Plant-Insect Interactions at Pollinator Habitats in Mead, NE

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Introduction

Beneficial insects play important roles in agriculture as pollinators, nutrient recyclers, and predators of pest insects but are declining globally for a variety of reasons, including exposure to pesticides that may kill and or disrupt ecosystem food webs as well as the balance between beneficial and pest populations[1]. The goal of this project was to provide a baseline understanding of the plant-insect interactions within conservation habitats as part of an ongoing study monitoring the effects of widespread systemic pesticide contamination from agricultural and industrial pollution on beneficial insect communities in Mead, NE. Efforts to improve pollinator health include the establishment of wild forage and corridor habitat or borders of diverse flowering plants near agricultural areas[2]. Sampling for this project took place in small habitat plots that were seeded with 53 species using Stock Seed Farms' Pollinator and Wildflower seed mixes[3] in the spring of 2017[4]. Therefore, the project further evaluated which seeded plants were still present and determined their potential value to beneficial insect communities after four years since planting and two years of minimal upkeep. Results will inform future monitoring efforts and provide insight to refine pollinator seed mix selections.

Methods

Figure 1. Pollinator habitat plot (left) and setting up linear transects for forb and insect surveys (right). This area is vulnerable to contamination from the nearby ethanol plant, which is located less than a mile upstream. Unusually high levels of neonicotinoid contamination have been detected in plants collected at ENREC (~2 to 3 miles downstream from the plant) in 2019 (*unpublished data*), suggesting that local insect populations may be at risk[5].

Forb and insect surveys conducted weekly over 5 weeks from mid-June through July 2021 at four pollinator habitat plots (~5x10 meters) located at the UNL Eastern NE Research and Extension Center (ENREC) in Mead, NE. This area is of particular concern due to the proximity to an ethanol plant, which had processed pesticide-treated seeds and highly contaminated waste byproducts for over 5 years less than a mile north of ENREC. (Figure 1).

- *Insects:* 15-min collection periods from blooming plants using aerial nets and vials along randomly selected linear transects (10 m) within each plot. Bees were identified to genus, and other insects were identified to family.
- *Forbs:* blooming plants were identified and quantified using 1 m^2 quadrat sampling tool at 4 random points along the same linear transect then pooled for each plot.

Results

- Of the 21 plant species observed in bloom, 14 (67%) attracted at least one insect and 9 (43%) attracted bees (Fig 2).
- Plants with higher bloom abundance generally attracted a higher number of insects, including bees (Fig 2).
- Three plants (bee balm, purple prairie clover, yellow prairie coneflower) supported mostly beneficial insects while other plants seem to be attractive to a broad range of insects, including pest species (Fig 3).
- Roughly 86% of total bees and 100% of represented bee genera were found on the top five plants (Fig 2,4) indicating lower diversity habitat plots may still be able to support a healthy and diverse community of bees.

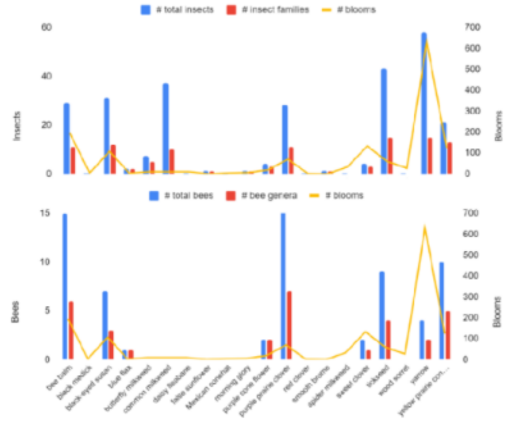


Figure 2: Graphs illustrate total cumulative bloom abundance (right axis), total cumulative insect abundance, and insect diversity per type of plant (top) as well as bee abundance and bee diversity (bottom) sampled over 5 weeks from mid-June through July 2021.

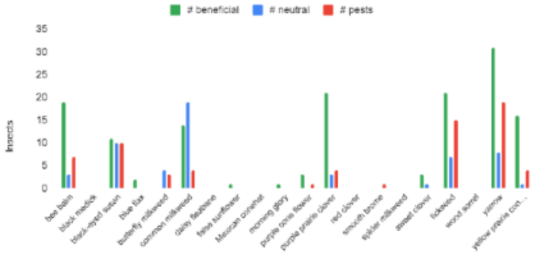
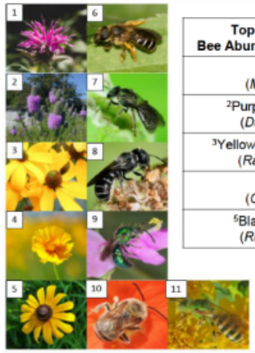


Figure 3: Graph illustrates cumulative totals of beneficial, neutral, and pest insects collected from each type of blooming plant. sampled over 5 weeks from June-July 2021. Insects were broadly categorized as "pests" if they are harmful to crops, "beneficial" if they are pollinators or predators of pests, and "neutral" otherwise.



Top Five Plants for Bee Abundance and Diversity	Top Five Bee Genera
[1]Bee Balm (*Monarda spp.*)	[6]*Halictus*
[2]Purple Prairie Clover (*Dalea purpurea*)	[7]*Lasioglossum*
[3]Yellow Prairie Coneflower (*Ratibida pinnata*)	[8]*Heriades*
[4]Tickseed (*Coreopsis sp.*)	[9]*Augochlorella*
[5]Black-Eyed Susan (*Rudbeckia hirta*)	[10]*Melissodes*, [11]*Agapostemon* (tie)

Figure 4. Table lists the 5 plant types on which the highest abundance and diversity of bees were found, and the 5 most abundant bee genera observed during forb and insect surveys conducted June-July 2021, with corresponding photos on the left.[6]

Discussion

- Results indicate many of the originally seeded plant species are no longer present and that pollinator plots could be seeded with fewer species but still maintain attractiveness to bees.
- Refining seed mixtures will reduce the cost of seed and provide cost savings with similar effects in the long run, since many seeded species are ultimately outcompeted by volunteer species and grasses.
- The five most abundant genera of bees were all solitary bees while only three social bees were collected in total (two *Bombus spp.* and one *Apis mellifera*). This surprising absence of social bees could be a topic for future research, as could comparisons between bees with different nesting habits and lifestyles.
- This data could also be compared to past and future research to see how insect populations are shifting and changing over time, particularly since widespread systemic pesticide contamination issues will likely persist in this area for several years to come.

Funding and References

Funding: This work is supported by the UNL Beneficial Insects REEU Program grant no. 2019-67013-29073 from the USDA National Institute of Food and Agriculture.

USDA National Institute of Food and Agriculture

1) Baltazar Ndakidemi, Kelvin Mtei, Patrick A. Ndakidemi (2016) Impacts of Synthetic and Botanical Pesticides on Beneficial Insects. *Agricultural Sciences*, 07,364-372. DOI: 10.4236/as.2016.76038
2) Long R, Corbett A, Lamb C, Reberg-Horton C, Chandler J, Stimmann M (1998) Beneficial insects move from flowering plants to nearby crops. *California Agriculture* 52(5):23-26. https://doi.org/10.3733/ca.v052n05p23.
3) *Wildflowers*. Stock Seed Farms. https://www.stockseed.com/Shop/wildflowers
4) Vakil, Surabhi Gupta (2020) *Landscape Enhancement Options to Reduce Pesticide Contamination in Pollinator Habitats and Increase Forage for Beneficial Insects in Agroecosystems*. UNL, PhD dissertation.
5) Shannon L. Bartelt-Hunt and Jesse E. Bell (2021) Surface and Groundwater Contamination, Community and Ecosystem Exposures Are the Unintentional Consequences from "Recycling" Treated Seed Products. *Environmental Science and Technology*, 55 (9), 5605-5607. DOI: 10.1021/acs.est.1c01305
6) Photos 1,3,4,5: https://pixabay.com/photos; photo 4: http://www.illinoiswildflowers.info/prairie/plants/ppr_clover.htm; photos 6,7,8,9,10,11: https://bugguide.net/

Nebraska University of Lincoln

Assessment of herbivory feeding by insects on milkweeds across Nebraska

Edith Ikuze & Dr. Judy Wu-Smart

University of Nebraska-Lincoln

Department of Entomology

Introduction

- The common milkweed (*Asclepias syriaca*) is an important and required host plant for monarch butterfly (*Danaus plexippus*) caterpillars which feed upon the leaves throughout their immature life stages.
- Milkweed flowers provide nectar and pollen for bees, butterflies, and other beneficial insects that provide critical ecological services, such as pollination, pest control by natural predators, and nutrient cycling necessary for sustaining soil health and water quality.
- Milkweeds growing near cropland may become contaminated by pesticides from spray applications, run-off, and uptake of systemic pesticide residues.
- Systemic pesticides, such as neonicotinoid insecticides and several types of fungicides are often used as crop seed treatments because residues may translocate to all parts of the seedling for full protection from root feeding and stem dwelling pests and pathogens. Unfortunately, harmful levels of residues may also be expressed in leaves, nectar, and pollen where beneficial insects may become inadvertently exposed.

Project Aim:
To assess insect herbivory or feeding damage of milkweed leaves collected at various locations around ENREC paired with pesticide residue analyses to determine if feeding damage may be utilized as useful indicators of systemic pesticide contamination.

Materials and Methods

- Milkweed plants were intensively collected between June-August 2021 at various locations across ENREC in Mead, and at two control sites (East Campus, Lincoln and Kimmel Orchard, NE City) (Figure 2).
- Milkweeds collected at ENREC sites were ~0.76 to 4 miles or (1.2 – 6.4km) away from the ethanol plant.
- Milkweed plants were divided evenly into top, middle, and bottom sections for leaf damage assessments and pesticide residue testing (*not shown*) (Figure 2C)
- Herbivory or insect feeding damage on leaves was quantified using the BioLeaf App (Figure 1).

Figure 1. Some leaves showed clear herbivory (A) while other leaves showed only slight damage marks (B). Each leaf was separately scanned to obtain a leaf area profile (indicated in black) and the relative feeding damage (shown in red) was calculated based on the total leaf area (C).

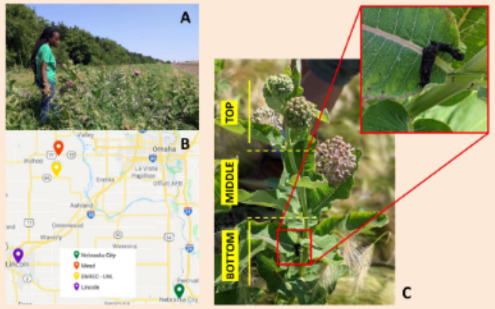


Figure 2: Sampling milkweeds adjacent to crop fields (A) across ENREC property in Mead, NE as well as two control locations (Lincoln and NE City) (B). Photo C illustrates how plants were partitioned into three sections (bottom, middle, top) for leaf damage assessments and monarch caterpillar found dead on the bottom section of the plant.

Results

- A total of 860 milkweeds were collected and assessed in 2021
- Insect visitors, included Monarch caterpillars, Milkweed beetles (*Tetraopes spp.*), and Milkweed bugs (*Oncopeltus fasciatus*). Insects on flowerheads were collected and will be identified and quantified to provide more information on milkweed feeding activity and visitation.

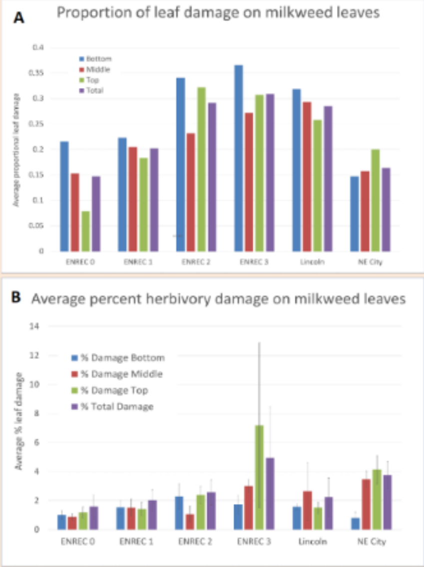


Figure 3. The proportion of leaves with any sign of feeding damage (A) and the average percent leaf damage (B) observed in milkweed leaves collected from the bottom, middle, and top sections of each plant. Milkweed plants were sampled randomly collected at sites <1 mile (ENREC 0), within 1 mile (ENREC 1), 2 miles (ENREC 2), and 3 miles (ENREC 3) away from the ethanol facility located in Mead, NE. Control sites included East Campus Gardens in Lincoln and Kimmel Orchard in Nebraska City.

Study sites

- The Eastern Nebraska Research and Extension Center (ENREC) in Mead, Nebraska is suspected to potentially have hotspots of contaminated milkweeds due to systemic pesticide pollution originating from waste run-off produced by an ethanol plant processing surplus outdated pesticide treated crop seeds as the primary source of carbohydrate for ethanol production (Figure 2B).
- Milkweed leaves collected on site in 2018-2019 yielded systemic neonicotinoids (clothianidin) levels ranging from 3,000 to 5,000 ppb associated with some but not all areas within ENREC.
- This project sought to investigate novels ways to identify "pesticide hotspots" in non-target plants using field scouting techniques for high mortality of insects and low herbivory or feeding damage.

Conclusions and discussion

- The preliminary results suggest there are differences in the proportion of leaves damaged across sections (bottom, middle, and top) (Fig 3A).
- Although bottom leaves had more signs of feeding, the % area damaged remained relatively consistent across sections (Fig 3B).
- Data also suggests plants collected within less than 1 mile or (<1.6 km) from the ethanol plant (ENREC 0) had lower feeding rates and less leaf damage compared to other plants collected farther away (Fig 3B).
- Milkweed leaves and flowerheads will be sent for pesticide residue testing to compare with observed feeding rates.
- Pollinators have been documented to prefer un-intoxicated plants with pesticides or other environmental pollutants (Olaya-Arenas, et al. 2020)
- Therefore, it is possible to assess feeding damage (or the lack of feeding activity) as a crude field monitoring technique to help researchers better locate potential "hotspots" that can be used to inform pesticide residue sampling and collection procedures for more accurate assessments of exposure and risk from systemic pesticide pollution.
- Future sampling and assessments of milkweeds and systemic pesticide contamination monitoring will continue through UNL Bee Lab program.

Acknowledgements

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- Special Thanks to Ihlara Gray for helping me with sampling and processing samples and the whole bee lab research team.
- IANR One Health Gap Funding Grant for partially funding the project
- The CUSP leadership team for their guidance throughout the whole research process.

References

1) Agrawal, A. (2017). *Monarchs and milkweed*. Princeton University Press.Hopwood, J. L. (2013, June). Roadsides as habitat for pollinators: management to support bees and butterflies. In *Proceedings of the 7th International Conference on Ecology and Transportation (IOECT): Canyons, Crossroads, Connections. Scottsdale, Arizona, USA* (pp. 1-18).Machado,B, Santos, M, Orue, J, & Viana,V. (2016). *BioLeaf*. Quantitative Plant | Quantitative Plant. https://www.quantitative-plant.org/software/bioleafOlaya-Arenas, P., Scharf, M. E., & Kaplan, I. (2020). Do pollinators prefer pesticide-free plants? An experimental test with monarchs and milkweeds. *Journal of Applied Ecology, 57*(10), 2019-2030.