2019 Milkweed Production Trials

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THE UNIVERSITY OF VERMONT
EXTENSION

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Milkweed (*Asclepias syriaca*) is a plant native to North America and has recently become the focus of conservation programs, as Milkweed is the sole food source for declining populations of Monarch butterfly larvae. Milkweed (Image 1) has long been a foe of agricultural operations and as a result, populations have been on the decline throughout the United States. To increase the abundance and scale of conservation plantings of milkweed, the Natural Resource and Conservation Service (NRCS) has developed an incentive program to compensate landowners for establishing perennial monarch habitat including planting milkweed. Landowners in northern Vermont have a unique opportunity to expand milkweed acreage by producing it as a crop. The silky fiber (floss) from the milkweed plant has a wide variety of oil/chemical absorbent and clothing applications. The floss has insulative properties similar to goose down due to its unique hollow fiber structure which also makes it incredibly light. Furthermore, the floss is equipped with a natural water-repellant waxy coating that allows it to be waterproof while absorbing hydrophobic liquids such as petroleum products. Producing milkweed as a crop will require farms to learn best techniques for cultivating milkweed versus the techniques they currently know which is to eliminate at first sight!


Image 1. Milkweed in bloom

Although milkweed is well adapted to a wide range of soils and growing conditions, economical commercial milkweed production has proven more difficult than initially anticipated. The main obstacle in production is weed pressure during the establishment year. Milkweed can be established during early summer in Vermont, making the slow-growing seedlings vulnerable to weed pressure from fast-growing annuals that are able to take advantage of lower temperatures early in the season. Furthermore, little is known about maintaining a milkweed stand for long-term production once it is established. In addition to these production challenges, little is known about how milkweed cultivation may impact soil microorganisms such as earthworms, beetles, ants, and mites. These organisms play a number of important ecosystem services in agricultural settings, including aiding in decomposition, soil structure creation, and pest control, but we do not know how these functions may differ in milkweed production systems. Furthermore, much of the research and conservation focus around insects has been focused on the monarch butterfly. However, we do not know what other species are utilizing milkweed plants or how milkweed production techniques may impact their populations or function. To support this emerging market and gain a better understanding of the impacts of milkweed cultivation, UVM Extension's Northwest Crops and Soils Program along with colleagues from the UVM Gund Institute and Plant and Soil Science Department, conducted three trials investigating best management practices for the establishment of milkweed.

MATERIALS AND METHODS

Milkweed fertility trials-nitrogen and potassium

Producing optimal yields of any crop requires having adequate levels of nutrients available in the soil. Typically, farmers will test their soils to determine their nutrient content and will receive recommendations on additional fertility required to produce an optimum crop. For milkweed, these required fertility levels have yet to be established. We hypothesize that, as with most crops, providing additional nitrogen will increase plant productivity. In addition, we hypothesize that, as with many deep tap rooted crops, milkweed productivity will increase with increased availability of potassium. However, with both of these, we do not know if the increase in productivity will translate into increased floss yield specifically, or if the level of supplemental fertilizer needed to attain the increased yield will be economical. To help determine optimal and economical nutrient management strategies that support a high yielding milkweed crop, two fertility trials, investigating rates of nitrogen and potassium, were established in 2019.

The experimental design in each trial was a randomized complete block design with four replications. Plots 8' x 35' were imposed into an area of milkweed that was established in 2016. Prior to the addition of fertilizer, the soil in the area was sampled to be analyzed for nutrient concentrations. Fertilizer treatments were hand applied on 7-Jun in both trials. At the time fertilizer was applied, all milkweed plants were in vegetative stages ranging from one to four pairs of leaves. Plots were also assessed for milkweed populations, height, and flowering status at the time the fertilizer treatments were implemented and again at harvest. Table 1 shows the treatments for each trial. The nitrogen was applied in the form of urea (46-0-0) while potassium was applied in the form of muriate of potash (0-0-60).

Table 1. Nitrogen and potassium treatments, 2019.

Nitrogen lbs N ac^{-1}	Potassium lbs K ac^{-1}
0	0
25	50
50	75
75	125
100	150

Image 2. Unripe seeds (left) ripe seeds (right)
(photo credit: Brianna Borders, The Xerces Society)

The plots did not receive any further management throughout the season. Determining the timing of milkweed harvest relies on two key factors: seed ripeness and pod opening. Milkweed pods are ready for harvest when the seeds inside ripen turning a brown color (Image 2). Plots were monitored for ripeness on a weekly basis by collecting a variety of pods from across the trial area and inspecting them for seed ripeness. To minimize floss losses during harvest, pods were harvested when the majority of seeds were ripe but before the pods had broken open. Plots in both trials were harvested on 16-Sep. At harvest, milkweed populations were determined by counting the number of plants within a 0.25m^2 quadrat. The number of the plants that had pods, and the total number of pods were recorded as well. Plant height and pod length were recorded for 5 randomly selected plants out of the quadrat area. The pods from the 5 plants were then weighed and a subsample dried to determine moisture content. A subset of the pods from each plot were also separated into pod, floss, and seed fractions and weighed.

Impact of herbicide use on milkweed stand productivity

Although weed pressure during establishment is known to be a challenge in successful milkweed production, we have yet to understand the impacts of weeds or weed management strategies over the stands' lifetime. As the stands fill in, there are little or no opportunities for mechanical cultivation. Many farmers are implementing chemical weed control in the spring prior to milkweed emergence. It is not clear if the application of chemical weed control is necessary and if there is an impact on milkweed yields. To investigate the impact of chemical weed control on milkweed productivity, an herbicide trial was implemented in a milkweed stand that was established in 2016. Prior to herbicide application, weed composition and ground cover were measured in each plot. This was done by visually identifying the weed species present in each plot and by using the beaded string method **(**Sloneker and Moldenhauer, 1977). On the 7-May a treatment of Roundup® was applied to treatment plots. Plots were 20' x 20' in area. Once the milkweed had emerged, plant populations were measured in each plot by counting the number of plants in two 0.25m^2 quadrats. The trial was harvested on 16-Sep. At harvest, milkweed populations were determined by counting the number of plants within a 0.25m^2 quadrat. The number of the plants that had pods, and the total number of pods were recorded as well. Plant height and pod length were recorded for 5 randomly selected plants out of the quadrat area. The pods from the 5 plants were then weighed and a subsample dried to determine moisture content. A subset of the pods from each plot was also separated into pod, floss, and seed fractions and weighed.

Variations in yield and quality can occur because of variations in genetics, soil, weather, and other growing conditions. Statistical analysis makes it possible to determine whether a difference among treatments is real or whether it might have occurred due to other variations in the field. All data was analyzed using a mixed model analysis where replicates were considered random effects. At the bottom of each table, a LSD value is presented for each variable (e.g. yield). Least Significant Differences (LSDs) at the 10% level (0.10) of probability are shown. Where the difference between two treatments within a column is equal to or greater than the LSD value at the bottom of the column, you can be sure in 9 out of 10 chances that there is a real difference between the two values. Treatments listed in bold had the top performance in a particular column; treatments that did not perform significantly worse than the top-performer in a particular column are indicated with an asterisk. In this example, treatment A is significantly different from treatment C, but not from treatment B. The difference between A and B is equal to 400, which is less than the LSD value of 500. This means that these treatments did not differ in yield. The difference between A and C is equal to 650, which is greater than the LSD value of 500. This means that the yields of these treatments were significantly different from one another.

Variety	**Yield**
A	**1600***
B	1200*
C	950
LSD (0.10)	500

RESULTS

Weather data was recorded with a Davis Instrument Vantage Pro2 weather station, equipped with a WeatherLink data logger at Borderview Research Farm in Alburgh, VT (Table 2). Growing Degree Days (GDDs) were summarized using the base and maximum temperatures for corn as they are not known for milkweed specifically. The season began cooler and wetter than normal however, periods of very hot dry weather were experienced mid-summer. In July, temperatures were consistently >80° F with little rainfall. The longest period without rain during this month was 12 days, about half as long as was experienced in 2018. These dry conditions occurred during pod and seed formation and therefore may have impacted development and productivity. However, these warm conditions did provide optimal Growing Degree Days (GDDs) through the season with a total of 2254 GDDs accumulated May-Sep, 42 above normal.

Table 2. 2019 weather data for Alburgh, VT.

Alburgh, VT	May	June	July	August	September
Average temperature (°F)	53.3	64.3	73.5	68.3	60.0
Departure from normal	-3.11	-1.46	2.87	-0.51	-0.62
Precipitation (inches)	4.90	3.06	2.34	3.50	3.87
Departure from normal	1.45	-0.63	-1.81	-0.41	0.23
Growing Degree Days (50-86°F)	189	446	716	568	335
Departure from normal	-9	-29	76	-13	17

Based on weather data from a Davis Instruments Vantage Pro2 with WeatherLink data logger.
Historical averages are for 30 years of NOAA data (1981-2010) from Burlington, VT.

Milkweed fertility trials-nitrogen and potassium

Fertility treatments did not significantly affect yield or many of the other harvest characteristics in either trial (Tables 3 and 4). The number of pods per plant averaged 2.85 and 2.84 with 60.4% and 48.9% of plants having formed pods in the nitrogen and potassium trials respectively. Pods averaged 9.49 and 9.86 cm in length and 57.7% and 58.8% moisture content at the time of harvest for the nitrogen and potassium trials respectively. The total pod yields, expressed on a dry matter basis, were 0.893 and 0.776 tons ac^{-1} for the nitrogen and potassium trials respectively. None of these characteristics varied statistically across nutrient application rates. Plant height also did not vary statistically in either trial averaging 106 cm and 97 cm in the nitrogen and potassium trials respectively. This result was surprising as we would assume that additional nitrogen would be utilized by the plant in its biomass. However, the wet conditions at the time of application followed by dry conditions throughout much of the season likely influenced the availability of nitrogen to the plants. Applying split applications of nitrogen may prove to be more beneficial since it would likely reduce the risk of loss to the environment.

Table 3. Milkweed harvest characteristics, nitrogen trial, 2019.

Nitrogen rate	Pod production		Pod length	Plant height	Pod moisture	Pod yield
lbs N ac^{-1}	pods plant^{-1}	% of plants	cm	cm	%	DM tons ac^{-1}
0	2.10	52.8	9.62	102	59.4	0.713
25	3.00	56.5	9.58	98.9	57.6	0.992
50	**3.12**	**86.1**	**9.58**	**113**	58.0	**1.25**
75	3.09	57.5	9.08	107	**55.4**	0.883
100	2.92	49.0	9.58	109	58.2	0.628
LSD (p = 0.10)	NS	NS	NS	NS	NS	NS
Trial mean	2.85	60.4	9.49	106	57.7	0.893

Treatments with an asterisk* performed statistically similarly to the top performer indicated in **bold**.
NS – Not significant.

Table 4. Milkweed harvest characteristics, potassium trial, 2019.

Potassium rate	Pod production		Pod length	Plant height	Pod moisture	Pod yield
lbs K ac^{-1}	pods plant^{-1}	% of plants	cm	cm	%	DM tons ac^{-1}
0	2.66	48.6	**10.2**	95.6	61.3	0.639
50	2.39	52.1	9.99	**103**	59.7	0.736
75	2.25	35.3	9.51	96.9	60.2	0.484
125	2.76	**72.6**	9.77	91.4	**55.7**	**1.21**
150	**4.13**	35.8	9.80	99.9	57.4	0.811
LSD (p = 0.10)	NS	NS	NS	NS	NS	NS
Trial mean	2.84	48.9	9.86	97.3	58.8	0.776

Treatments with an asterisk* performed statistically similarly to the top performer indicated in **bold**.
NS – Not significant.

Treatments also did not differ significantly in terms of pod composition across either trial (Tables 5 and 6). The majority of the total pod weight is composed of external pod cover as this was found to be 59.9% and 60.2% for the nitrogen and potassium trials, respectively. The floss, as to be expected, accounted for the smallest fraction at only 17.4% and 16.9% of the total pod weight for the nitrogen and potassium trials, respectively. Based on the pod yields observed in the trials, and the current value estimate for pods at 30% moisture being $0.40 per pound, the value of the crop would be between $700 and $1400 per acre. However, the actual value that can be realized may be lower as these estimates assume that all of the pods can be harvested from the field without loss.

Table 5. Milkweed pod composition by weight, nitrogen trial, 2019.

Nitrogen rate	Floss	Pod	Seed
lbs N ac^{-1}			% by weight
0	16.6	61.6	21.8
25	15.3	60.2	**24.6**
50	18.8	58.4	22.8
75	**19.1**	**57.8**	23.1
100	17.3	61.3	21.5
LSD (p = 0.10)	NS	NS	NS
Trial mean	17.4	59.9	22.7

Treatments with an asterisk* performed statistically similarly to the top performer indicated in **bold**.
NS – Not significant.

As with any crops, some level of loss at harvest is to be expected, however, it is exceptionally high with milkweed given the extremely low weight of the floss. Harvesting techniques to minimize floss losses and improve purity and cleanliness are currently being developed. Although the floss is the main component of interest in a milkweed crop, the seed may also present opportunities to recoup value, especially as interest in growing milkweed commercially increases.

Table 6. Milkweed pod composition by weight, potassium trial, 2019.

Potassium rate lbs K ac^{-1}	Floss	Pod	Seed
			% by weight
0	16.6	62.2	21.1
25	17.4	61.9	20.7
50	**17.8**	58.6	23.6
75	16.6	60.7	22.7
100	15.8	**57.4**	**26.8**
LSD (p = 0.10)	NS	NS	NS
Trial mean	16.9	60.2	23.0

Treatments with an asterisk* performed statistically similarly to the top performer indicated in **bold**.
NS – Not significant.

Impact of herbicide use on milkweed stand productivity

Weed control treatments did not significantly affect yield and most harvest characteristics (Table 7). The number of pods per plant averaged 2.41 with 60.2% of plants on average having pods. Pods averaged 9.29cm in length and plants averaged 75.8cm in height at the time of harvest. The total pod yield, expressed on a dry matter basis, was 0.768 tons ac^{-1}. While none of these characteristics varied statistically across weed control methods, ground cover from weeds, an estimate of weed pressure, was significantly higher in the control plots than the plots receiving the herbicide application (Table 8). Interestingly, the reduction in weed pressure did not ultimately translate into higher milkweed populations or productivity. This suggests that herbicide application is likely not cost effective in established milkweed stands.

Table 7. Milkweed harvest characteristics, weed control trial, 2019.

Weed control	Ground cover	Pod production		Pod length	Plant height	Pod moisture	Pod yield
	%	pods plant^{-1}	% of plants	cm	cm	%	DM tons ac^{-1}
Herbicide	**45.0**	2.00	52.7	8.84	75.1	62.3	**0.916**
Control	76.7	**2.83**	**67.6**	**9.63**	**76.4**	**60.5**	0.657
LSD (p = 0.10)	27.5	NS	NS	NS	NS	NS	NS
Trial mean	60.8	2.41	60.2	9.29	75.8	61.4	0.768

Treatments with an asterisk* performed statistically similarly to the top performer indicated in **bold**.
NS – Not significant.

Table 8. Milkweed pod composition by weight, weed control trial, 2019.

Weed control	Floss	Pod	Seed
			% by weight
Herbicide	**18.2**	**61.1**	20.7
Control	14.7	62.5	**22.8**
LSD (p = 0.10)	NS	NS	NS
Trial mean	16.5	61.8	21.7

Treatments with an asterisk* performed statistically similarly to the top performer indicated in **bold**.
NS – Not significant.

However, overall weed pressure at the research site has remained relatively low over the course of the stands' lifespan and therefore, if higher weed pressure is present, a growth or yield benefit may be seen thereby impacting the economics of this practice.

DISCUSSION

These preliminary data suggest that additional nitrogen or potassium fertilizer at rates between 0-100 and 0-150 lbs ac^{-1} respectively do not increase milkweed floss yield. The soil test results from the test field indicated levels of soil K considered optimum for most field crops and therefore, fields with very low soil test levels of K may experience a greater yield response to additional fertility applications. In terms of nitrogen, lack of moisture throughout the season likely contributed to lower nitrogen availability in the soil. Lastly, it would be important to evaluate timing of fertility applications to milkweed. Like most crops, greater amounts of nutrients are required as the plant builds biomass and shifts to the reproductive stage. Later applications of nutrients might have a larger impact on milkweed pod yields more so than early spring applications. These data also suggest that one singular spring application of herbicide did not increase milkweed floss yield despite lowering weed pressure. These data are representative of only one location and year. Further investigation is needed to determine optimal and economical fertility rates and weed control methods for milkweed.

REFERENCES

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COMMERCIALLY GROWN MILKWEED AS HABITAT AND FORAGE FOR MONARCH BUTTERFLIES AND OTHER POLLINATORS

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The decline of pollinating insect species including monarch butterflies and some bee species is partly attributed to the loss of forage and habitat associated with land use change (Kremen, Williams & Thorp, 2002; Potts et al., 2010; Winfree, Bartomeus & Cariveau, 2011). As a result, in recent years, there has been a concerted effort to create 'pollinator friendly' gardens and hedgerows of flowering plant species and larval host plants (Morandin & Kremen, 2013; Kim et al., 2006; Pollinator Health Task Force, 2015). Milkweed is an important source of nectar for bees and is the larval host plant of the monarch butterfly (*Danaus plexippus*). Previous research suggests that a loss of agricultural milkweed is a major contributor to the decline of the monarch population (Pleasants & Oberhauser, 2013). A new opportunity to grow and harvest milkweed at the commercial scale has the potential to create large areas of habitat and forage for monarch butterflies and bee species. As our research group and Vermont farmers develop management practices such as IPM strategies and harvesting techniques, it is important to note how these practices coincide with monarch butterfly life cycle and pollinator activity. The development of sustainable management practices for milkweed crops requires an understanding of pollinator activity on milkweed throughout the growing season to reduce any potential negative impacts on pollinator species.

Agricultural chemicals such as pesticides also pose a threat to pollinating insect species (Goulson et al., 2015). In particular, neonicotinoids, a class of systemic pesticides widely used in agriculture and applied as seed dressings on crops, have garnered much attention in recent years. Negative impacts to many pollinator groups are well documented and include butterflies, bumble bees, solitary bees, and honey bees (Cresswell, 2011; Hopwood et al., 2013; van der Sluijs et al., 2013). Field and laboratory experiments suggest that neonicotinoid exposure to adult bees at field realistic doses impairs foraging activity, increases worker mortality, reduces queen production, and weakens bee's immune system (Gill & Raine, 2014; Rundlöf et al., 2015; Arce et al., 2017; Baron et al., 2017; Fauser et al., 2017; Stanley & Raine, 2017; Woodcock et al., 2017). Monarch larvae that ingest milkweed contaminated with clothianidin, a neonicotinoid used in treated corn, experienced sub-lethal and lethal effects (Pecenka & Lundgren, 2015).

Pollinators may become exposed to neonicotinoids through multiple routes. As systemic pesticides, neonicotinoids are expressed not only in leaf tissue of plants but also in pollen and nectar of flowers, where floral visitors may become exposed when foraging (Goulson, 2013; David et al., 2016). Hedgerows of wildflowers grown adjacent to treated crops such as corn can be contaminated by wind-blown dust during sowing or pollen during crop bloom (Botías et al., 2015; Krupke et al., 2017). Due to their persistence in soils, neonicotinoids may also contaminate non-target plants when planted directly into soil that has previously supported a crop grown from neonicotinoid-treated seed (Basley & Goulson, 2018). It is unclear whether commercial milkweed crops, which have the potential to support pollinator communities, could become contaminated with neonicotinoids when planted near treated crops or in soil that previously supported a treated crop. More research is needed to test for contamination potential to milkweed crops near and around treated crops. Results could inform management practices to reduce impact to pollinators that use this plant as a host plant or source of nectar.

We conducted a two-year study to determine pollinator presence within milkweed crops as well as the potential for contamination of neonicotinoids to milkweed from treated corn. Over the course of two years, our aims were:

1. Determine the density and presence of monarch (eggs, larvae, and adults) throughout the growing season to inform management/growing recommendations for commercial milkweed crops.
2. Examine the diversity and abundance of common milkweed pollinators with a focus on bees.
3. Measure the presence of milkweed pests in commercial milkweed crops.
4. Test whether milkweed plants can 'take up' or sequester neonicotinoids from soil that was previously planted with treated corn seed.

Results from this study will define the potential for commercial milkweed crops to support pollinator communities and also guide sustainable management practices for this crop.

MATERIALS AND METHODS

In 2018 and 2019, we conducted surveys at two field sites with commercially planted common milkweed (*Asclepias syriaca*): Borderview farm (45.008689, -73.309507) and Dewing farm (44.980554, -72.843054). The plot sizes of milkweed crops at the Borderview and Dewing sites are 14 hectares and 6 hectares, respectively. In 2018, at each site, we delineated three separate blocks each consisting of 10,000 m^2 for the monarch/pollinator surveys. In 2019, due to differences in the size of the two milkweed plots and the apparent differences in monarch presence at the two sites from the 2018 data, we increased our sampling effort to include five separate blocks at Borderview and three blocks at Dewing. In both 2018 and 2019, to estimate milkweed plant density, we counted all milkweed plants within a one square meter quadrat randomly placed 100 times throughout each block for a total of 300 quadrates for each site.

To measure monarch (egg, larvae, adult) presence throughout the milkweed plots, we conducted weekly surveys (2018: 12-Jun – 3-Sep; 2019: 12-Jun – 8-Sep). During each survey event, we randomly selected 100 plants within each of the delineated blocks and inspected each plant for monarch presence. We recorded all eggs, larvae (and instar), and adults (female/male). In 2019, each plant that was examined for monarch presence was also examined for the following milkweed pests: red milkweed beetle (*Tetraopes* spp.), large milkweed bug (*Oncopeletus fasciatus*), small milkweed bug (*Lygaeuc kalmia*) and orleander aphid (*Aphis nerii*). We surveyed each pest weekly according to established protocols (Xerces Society for Invertebrate Conservation, 2017), focusing on a suite of pests according to milkweed phenology and when each pest species cause damage to the plant (Table 1). During pre-bloom and bloom, we estimated the number of aphids located on an 8-inch terminal growth point per plant. During the entire season (pre-bloom, bloom, and pod formation), we recorded the presence of beetles and when present, estimated the % of defoliation from leaf feeding and performed a 'tug test' of the plant to scout for larval presence. During pod formation, we surveyed for milkweed bugs and estimated the percentage of developing pods with milkweed bugs present.

Table 1. Pest scouting was conducted in milkweed plots according to milkweed phenology. Shaded areas indicate when a particular pest was scouting according to milkweed phenology (pre-bloom, bloom, and pod formation).

Milkweed phenology->	Pre-Bloom	Bloom	Pod Formation
Milkweed Beetles->	shaded	shaded	shaded
Milkweed Bugs ->			shaded
Aphids ->	shaded	shaded	

To measure pollinator diversity and abundance, we conducted weekly observational and collection surveys during milkweed bloom for 2018 and 2019. For observational surveys, we randomly selected two 50 meter transects within each block and recorded all floral visitors along this transect for 10 minutes on both milkweed flowers and other wildflowers ('weeds'). Floral visitors were identified to morphospecies: *Bombus* (queen), *Bombus* (worker), honey bee, black (big), black (slender), black (tiny), and green. To estimate pollinator diversity, we made collections of floral visitors (with a focus on bees) along two randomly selected 50 meter transects within each block for 10 minutes. All collected specimens were sacrificed, pinned, labeled, and identified to genus or species when possible. In 2018, pollinator surveys were conducted 26-Jun, 3-Jul, and 10-Jul at both sites. In 2019, pollinator surveys were conducted at both sites on 10-Jul and 18-Jul, and at Dewing only on 24-Jul.

To test neonicotinoid residues in the soil and milkweed leaf tissue, we selected soil (~500 g) and milkweed leaf tissue (~50 g) samples from Borderview. Sampling protocols varied between 2019 and 2019. In 2018, samples were collected on 24-Sep (two milkweed samples) and 3-Oct 2018 (21 milkweed samples and 21 soil samples). We initially attempted to collect leaf tissue from milkweed seedlings earlier in the season but growing conditions and low germination rates prevented earlier collections. In 2018, we collected four different types of samples: *active corn*: from fields planted with corn in 2018; *milkweed field*: where milkweed has grown for three years but corn was previously planted for three continuous years prior to milkweed; *new field*: where milkweed was seeded in spring of 2018 and grown beside corn; *new field no corn*: where milkweed was seeded in spring of 2018 but outside of corn plantings.

In 2019, milkweed and soil were collected from three areas according to when corn was last planted: a field where corn was planted in 2019 and actively growing, and two fallow plots where corn had been planted in 2015 and 2018. From each of the three areas, seven samples of milkweed (~50 g) and seven samples of soil (~500 g) were collected. In 2019, all samples were collected on 24-Jul to coincide with peak monarch presence.

All soil and milkweed samples were collected and stored on -80° C until further analysis. Samples will be analyzed for three neonicotinoid compounds (clothianidin, thiamethoxam, and imidacloprid) at the Vermont Agency of Agriculture laboratory at the University of Vermont.

RESULTS AND DISCUSSION

In 2018, milkweed density at Borderview was twice that of Dewing, (Borderview: 24.25 plants m^2; Dewing: 12.42 plants m^2). In 2019, milkweed density was similar between the two sites (Borderview: 26.11 plants m^2; Dewing: 26.10 plants m^2). This difference is likely due to the use of herbicides by the farmer at Dewing to reduce weed pressure in 2019. Overall, milkweed bloom lasted from 26-Jun to 10-Jul in 2018 and 10-Jul to 24-Jul in 2019. Phenology of milkweed bloom at Borderview was approximately one week before Dewing for both years. Although we did not conduct formal wildflower surveys throughout the fields, we anecdotally note that Dewing had a much higher density of wildflowers throughout the milkweed plots compared to Borderview, especially in 2018, that supported an abundance of bees. Thus, management strategies for milkweed crops should also consider the presence of wildflowers dispersed throughout the fields and the pollinator communities they support. In the future, formal wildflower surveys should be conducted to examine the importance of these plants on the pollinator community within milkweed crop fields.

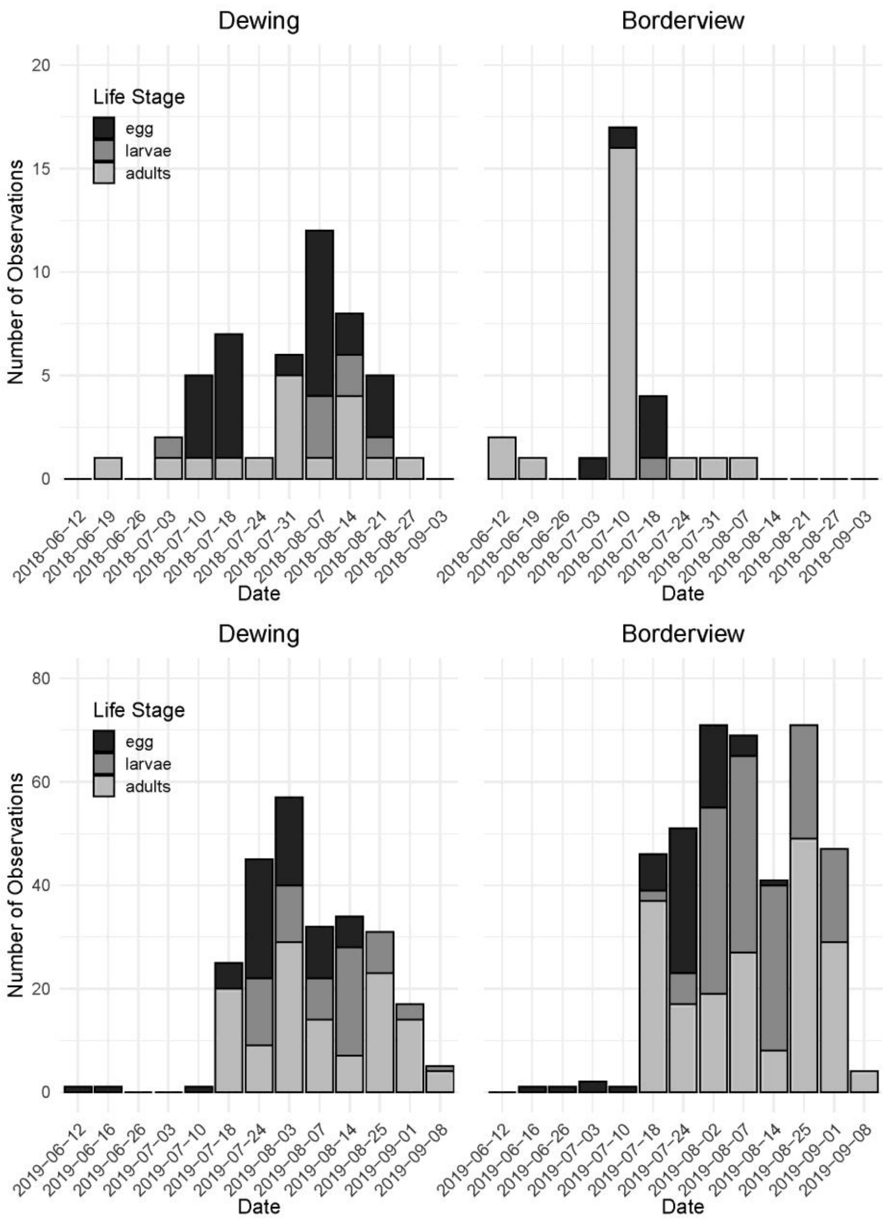

Figure 1. Monarch presence for each site throughout the sampling periods of 2018 and 2019.

In 2018, we observed a total of 7704 plants for monarch presence (3800 at Dewing and 3904 at Borderview). In 2019, we observed a total of 10,450 milkweed plants for the presence of monarchs and pests (3950 at Dewing and 6500 at Borderview).

We observed monarch eggs, larvae and adults at both Dewing and Borderview sites throughout the sample period in both 2018 and 2019 (Figure 1). In 2018, we observed a higher overall presence of monarchs at Dewing compared to Borderview. Observed differences in monarch activity between the two sites in 2018 may have been an artifact of sampling effort combined with differences in plot size and/or milkweed density. In 2019, we increased sampling effort at Borderview to include five survey plots to better capture monarch presence at this site. In 2019, we observed significant monarch presence at both sites. Phenology was shifted in 2019 compared to 2018, which greatly affected the date when monarch activity waned, which could have implications for when milkweed should be harvested to avoid monarch mortality. In 2018, monarch presence waned significantly by the end of August. In 2019, monarch presence was observed through the first week of September. Due to annual differences in phenology, we suggest annual scouting for monarch presence prior to milkweed harvest through the first few weeks of September.

In 2018 and 2019, we surveyed both sites weekly during milkweed bloom for pollinator presence with a focus on bees. During observational surveys in both years, honey bees (*Apis mellifera*) were the most predominant visitor of milkweed flowers followed by worker bumble bees (*Bombus* spp.) In the collection surveys, collections consisted of bees from the following genera: *Bombus*, *Augochlora*, and *Melissodes* with *Bombus* being the most predominant (Figure 2). Notable sightings included *B. terricola* (four in 2018 and 12 in 2019), and a species listed as state threatened in 2015, and one *B. fernaldae* (collected at Dewing on July 10th, 2018), representing the forth record of this species in Vermont.

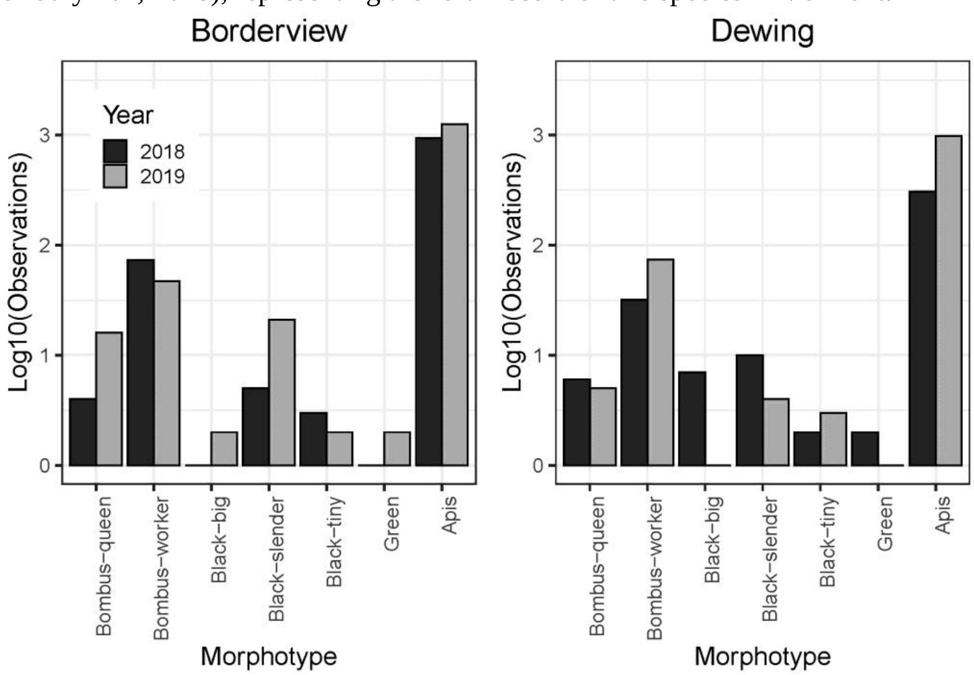

Figure 2. Observational surveys of floral visitors in Dewing and Borderview milkweed crops for 2018 and 2019.

All floral visitors to milkweed were recorded by morphotype. Observations have been log10 transformed for visualization.

Based on pest scouting results, presence for all pests were below recommended threshold for treatment. Aphid presence peaked during the pre-bloom period (Figure 3). The average number of aphids across the entire sampling period was 0.20 aphids/8 inches of terminal growth (sd: 0.91). The maximum number of aphids was 20 per terminal growth point. Xerces recommends considering further action when the average number of aphids per terminal growth point exceeds 50 aphids. We did not observe any plants with aphid levels that would require further action.

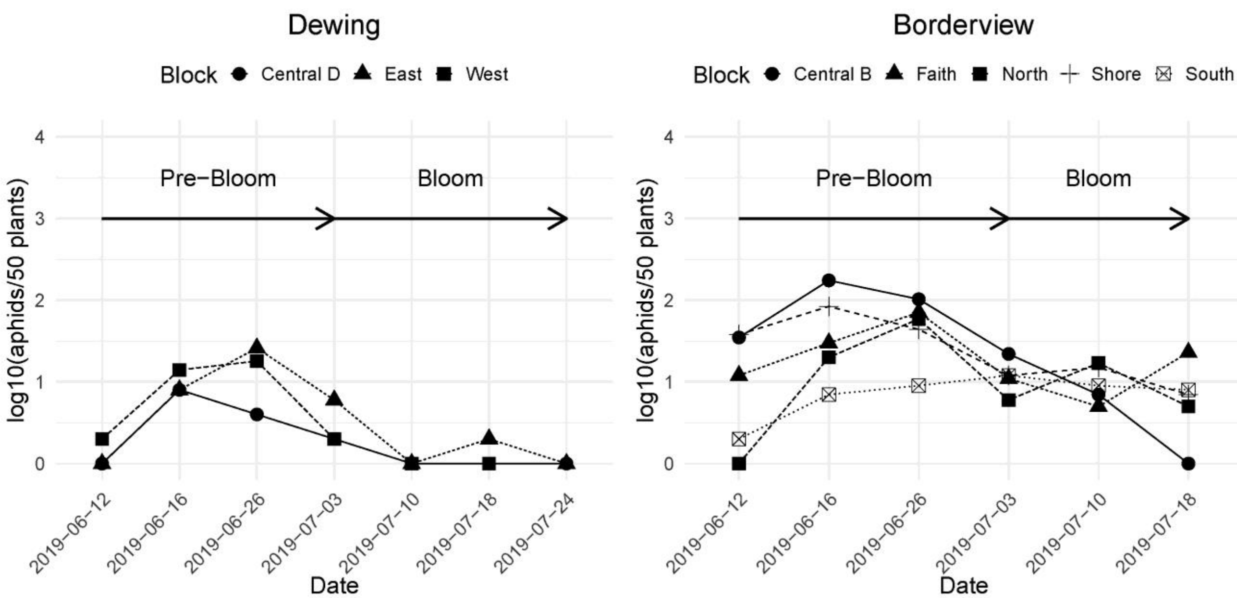

Figure 3. Aphid presence on milkweed plants throughout the pre-bloom and bloom periods by block. Observations have been log10 transformed for visualization.

Overall, beetle presence was low across both sites but lowest in Dewing compared to Borderview (Figure 4). For plants with beetles present, the average percent defoliation as a result of adult beetle feeding was 0.4% (sd: 1.5%). The maximum percent defoliation we observed was 20%. Xerces recommends considering taking further action only when beetles are observed, and defoliation exceeds 25% on a majority of plants scouted. We only observed four plants with milkweed bugs. Each of these plants had only one pod with a milkweed bug. We did not observe any plants with beetle or bug presence that would require further action.

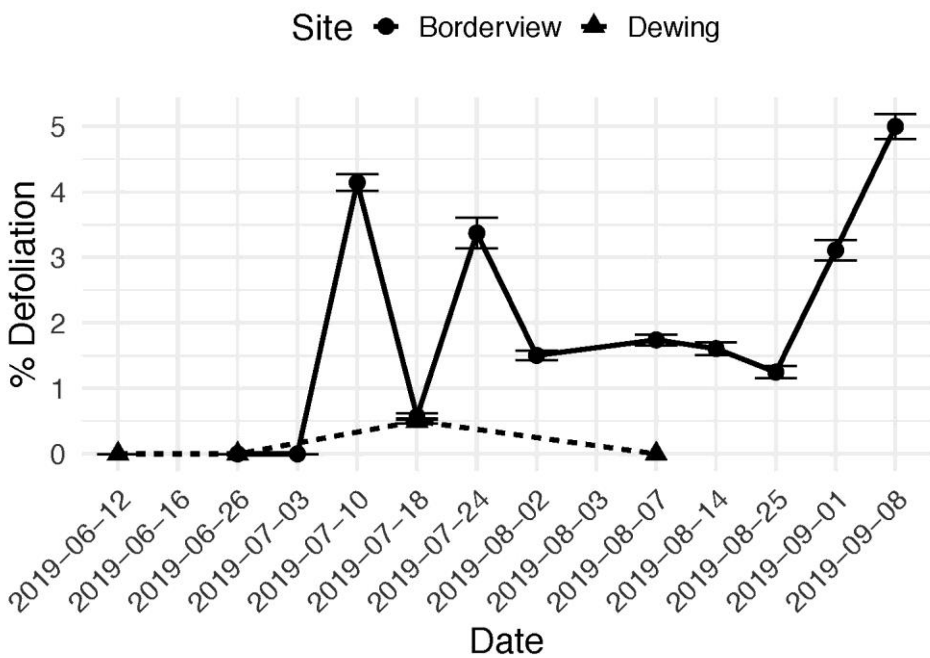

Figure 4. Percent leaf defoliation of plants with beetles observed.

Results from pesticide testing are not yet available from the Vermont Agency of Agriculture Laboratory. The Agency's lab was relocated from Burlington to Randolph in 2019, which resulted in delays. We expect results will be made available within the next six months.

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IMPACT OF MILKWEED CULTIVATION ON SOIL INVERTEBRATE COMMUNITIES

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Growing milkweed may be beneficial to monarch butterflies, but what impact does it have on other organisms? Soil invertebrates include organisms like earthworms, beetles, spiders, ants, springtails, and mites. This group plays a number of important ecosystem services in agricultural settings, including aiding in decomposition, soil structure creation, and pest control (Lavelle et al. 2006), and high diversity of these organisms can indicate healthy soil (Stork & Eggleton 1992). Agricultural management, such as tilling, mono-cropping, and pesticide use, has on many accounts shown to negatively impact soil invertebrate communities (i.e. House & Parmelee 1985). Milkweed, which does not have a history of domestication similar to other crops and is perennial (and thus has minimal tilling needs), might harbor more diverse and abundant soil invertebrate communities than other common agricultural land covers. In this project, we sought to understand how soil invertebrate communities differ under milkweed cultivation as compared to natural forest cover, and the two most common crops in Vermont, corn and hay. We hope that unearthing the impact of agriculture on this community can help guide management that preserves soil invertebrate diversity and functionality.

METHODS

At Borderview Research Farm in Alburgh, VT, 100 meter transects were established in nearby fields of milkweed, conventional corn, hay (perennial forage grasses), and forest. Sampling was conducted every 10 meters along the 100 meter transects (10 sampling sites per land use).

At each sampling site we used three methods to broadly survey soil invertebrates. The first method, pitfall traps, targets aboveground and litter-dwelling organisms. In this method, collection cups are placed in the soil with their lids level to the surface, and organisms randomly fall and are trapped in the cups as they move across the soil surface (Southwood & Henderson 2009, p. 276). The second method, the Berlese funnel method, targets belowground organisms. For this method, we extract organisms from soil cores by placing soil in a funnel and exposing it to a bright light from above (Southwood & Henderson 2009, p. 229). Organisms avoid light's heat and dryness by crawling downward and are funneled into a collection cup. Finally, in our third method we used mustard powder solution to specifically target earthworms (Lawrence & Bowers 2002). We poured the mustard solution (1 gallon of water with 1/3 cup mustard powder) on a 0.5m x 0.5m section of soil and collected earthworms that emerged within fifteen minutes. Earthworms evacuate the soil because they are irritated by the mustard powder. We chose this method because it has minimal impact on the field, unlike other methods that involve excavating large sections of soil and manually counting earthworms. We additionally sampled temperature and moisture at four sites along the transects. We sampled these physical soil measurements both in the morning (8am to 9am) and in the afternoon (1pm-2pm) on one sunny day in June.

This experiment was carried out over the summers of 2018 and 2019. In 2018, we took preliminary soil measurements and sampled using pitfall traps, Berlese funnels, and the mustard method. In 2019, we repeated the mustard experiment and took more extensive soil measurements.

All collected organisms were identified to at least to the taxonomic order level and measured for weight, length, and width. Identification was aided by a number of resources, but especially the textbook *Soil Biology Guide* (Dindall 1990) and the free online site iNaturalist, where posted photos of organisms are identified by the public. The link to this project's iNaturalist collection is here: https://www.inaturalist.org/projects/soil-organisms-of-alburgh-vt.

For each plot, we calculated the relative abundance of individuals from common orders, order richness (number of taxonomic orders), and total number of individuals captured. We additionally calculated Shannon's diversity (an index that takes into account the evenness of order representation) and size distribution of organisms in each land use type, though those results are not presented here.

RESULTS

Physical soil measurements: Forest soils had the coolest temperatures, with little change between the morning and afternoon (Table 1). Milkweed and hay soils had intermediate temperatures, and corn soils were both the hottest and the driest in both in the morning and the afternoon. Hay plots were the moistest in the morning, but lost moisture by the afternoon, while forest plots had intermediate moisture in the morning but gained moisture by the afternoon.

Table 1: Physical soil measurements between treatments, with averages and standard error. Letters denote significance within rows: values with matching letters are not significantly different.

		Corn	Hay	Milkweed	Forest
Temperature (°F)	Morning	69.4 ± 1.3[a]	59.1 ± 0.5[b]	64.3 ± 1.9[c]	53.9 ± 0.3[d]
	Afternoon	81.6 ± 1.0[a]	67.2 ± 0.4[b]	77.2 ± 0.2[c]	55.4 ± 0.5[d]
Moisture (volumetric water content % x100)	Morning	2576.0 ± 28.5[a]	3000.8 ± 116.3[b]	2735.0 ± 48.1[ab]	2650.0 ± 45.6[a]
	Afternoon	2423.3 ± 24.1[a]	2704.2 ± 104.6[b]	2735.8 ± 39.4[b]	2864.2 ± 39.7[b]

Pitfall traps (aboveground organisms): Conventional corn plots were dominated by larger ground beetles (specifically of the Harpalini Tribe), while hay plots had the highest number of ants and forest plots had the highest number of harvestmen spiders. Milkweed plots were relatively dominated by true spiders and crickets (Figure 1). Milkweed pitfall traps additionally had significantly higher order richness than the other treatments (with on average six taxonomic orders in each plot), and milkweed and hay had the highest average number of individuals per plot (around 25 individuals) (Figure 2).

Berlese funnels (belowground organisms): Similar to the pitfall traps, the Berlese funnel method demonstrated a clear difference in soil invertebrate communities among land cover types. Conventional corn plots had the highest average number of poduromorpha springtails, while milkweed had the highest number of elongate-bodied springtails. Forest plots had the most mites (both oribatid and mesostigmata) and the most symphyla (a small centipede-like organism) (Figure 1). Order richness was highest in forest plots and lowest in corn and hay. Similarly, the total number of individuals extracted in each plot was highest in forest and lowest in corn (Figure 2).

Mustard method (earthworms): Very few earthworms were extracted in 2018 and 2019, with most plots yielding no worms. Even with extremely low numbers, forest plots had significantly more worms than other land cover types (average of 0.7). Worms were also captured in the other methods (pitfall and Berlese), and aggregating data from all three methods shows a relative abundance of earthworms in forest and milkweed plots (Figure 3).

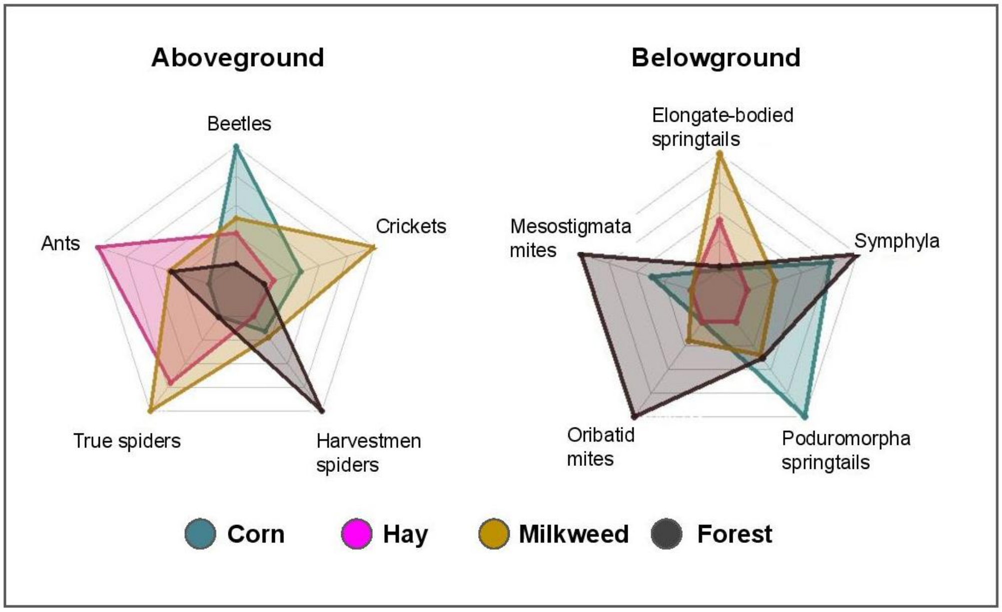

Figure 1: Relative abundance of individuals from common orders among land cover types for both aboveground (pitfall traps) and belowground (Berlese funnel) organisms.

DISCUSSION AND NEXT STEPS

These results demonstrate that milkweed hosts a diverse community of both aboveground and belowground soil invertebrates, performing as well or better than forest plots in most analyses. Conventional corn plots stood out as having the least diverse soil invertebrate community, with plots dominated by one ground beetle species. Low diversity in the corn plots is most likely due to intensive and frequent practices of tilling and herbicide use there. While hay plots performed moderately well in most analyses, we were surprised by the difference between hay and milkweed, because both are perennial. These differences may be due to increased litter biomass in milkweed plots, which serves as habitat or a food source for soil invertebrates.

Combining three sampling methods offered a broad view of the soil invertebrate communities and demonstrated key differences among land uses. In particular, the sampling methods demonstrated interesting differences between aboveground and belowground communities. While forest plots had relatively low diversity of aboveground organisms, they had the highest richness of belowground organisms. This likely reflects the history of minimal disturbance in the forest plots (as contrasted by the highly disturbed agricultural treatments), which has allowed a diverse and abundant belowground soil community to develop and persist. There may be less aboveground organisms in the forest plots because the dominant plant type there is large trees, with minimal understory plant life.

In the next phase of this project, we plan to include and analyze information on organism functional traits, such as body size, mouth parts (i.e., chewing, piercing-sucking, siphoning), and feeding groups (i.e., predator, detritivore, herbivore). This will allow us to assess differences in the soil food web between treatments, for instance understanding whether a treatment is dominated by herbivore (potentially pest) species. This may also allow us to understand what kinds of ecosystem services the soil invertebrate community provides in each land use.

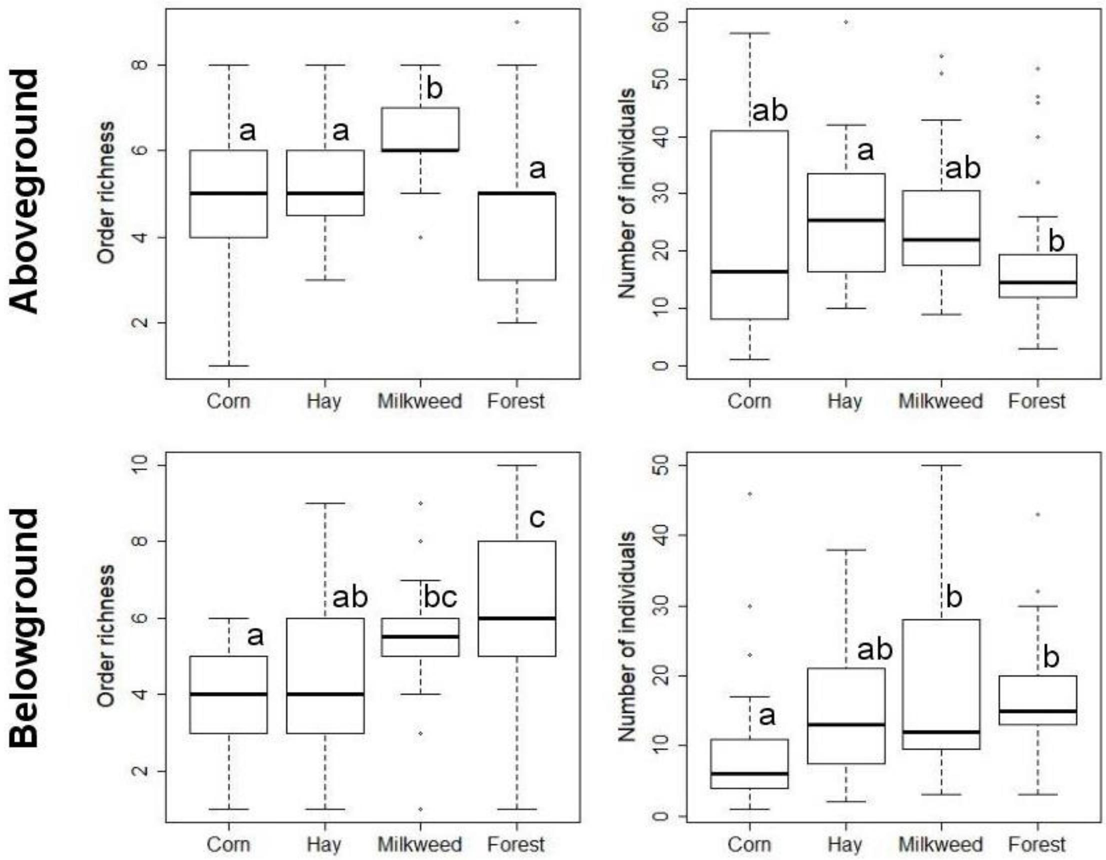

Figure 2: Order richness (number of orders represented in each plot) and number of collected individuals per plot among land covers. “Aboveground” organisms were caught using the pitfall method and “belowground” organisms were extracted using Berlese funnels. Letters denote significance: land covers with matching letters are not significantly different.

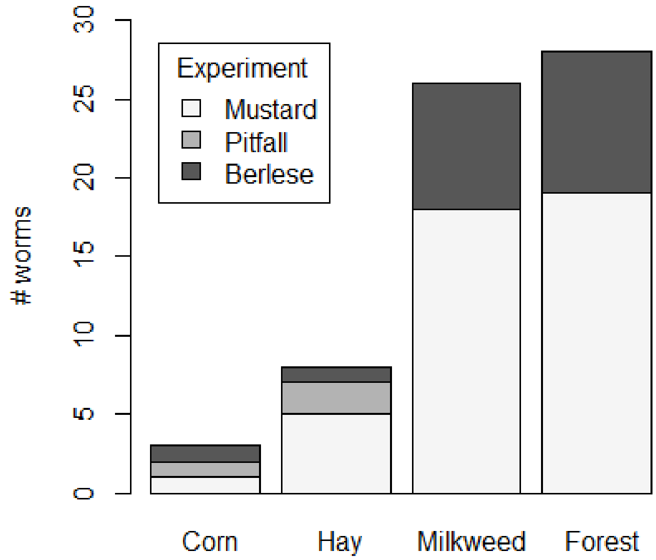

Figure 3: Total number of worms collected in 2018 and 2019 in all three methods.

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