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Research

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Pest Management

Weight Gain of *Spodoptera frugiperda* Larvae (Lepidoptera: Noctuidae) on Leaf and Floral Tissues of *Silphium integrifolium* (Asterales: Asteraceae) Differs by Plant Genotype

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Abstract

Silflower (*Silphium integrifolium* (Michaux)) is a native North American relative of sunflower that is undergoing domestication as a perennial oilseed crop. As silflower incurs pest damage from multiple insect species, it is necessary to screen genotypes for their effect on insect performance such that more pest tolerant/resistant accessions can be incorporated into future silflower breeding programs. We present a bioassay protocol for silflower using the generalist herbivore fall armyworm (*Spodoptera frugiperda* (J. E. Smith)). In this study, fall armyworm larvae were placed on leaf and flower tissue from eleven silflower genotypes, one cup plant (*Silphium perfoliatum* (L.) (Asterales: Asteraceae)) genotype, and an inbred sunflower line (*Helianthus annuus* (L.) (Asterales: Asteraceae), HA89). Caterpillar weight gained during a 4-d feeding period significantly differed on leaf and floral tissue from different silflower genotypes, between the *Silphium* species (silflower and cup plant), and between *Silphium* genotypes and annual sunflower. Two wild silflower genotypes produced lower larval weight gain on both the floral and leaf tissue than all other genotypes, suggesting these genotypes have either lower nutrition or greater resistance to fall armyworm. However, nonsignificant correlations between larval growth on floral versus leaf tissue across all plant species tested and among all silflower accessions suggest insect performances on these tissue types in silflower are independent. Along with identifying germplasm of interest for silflower breeding programs, we established an easily replicable bioassay protocol using fall armyworm on silflower floral and leaf tissues.

Key words: fall armyworm, bioassay, domestication, silflower, Theil-sen regression

When domesticating a new crop species, it is crucial to ensure that the crop species retains adequate pest defenses. Defenses may enable plants to resist (Rasmann and Agrawal 2009) or tolerate (Strauss and Agrawal 1999) the damage caused by a myriad of pests and diseases that cost billions of dollars in losses and negatively impact farmers' livelihood (Savary et al. 2019). A necessary first step to breed for crop resistance and tolerance to insect pests is a sound understanding of the variation available to breeders. Although characterizing diverse germplasm can be time intensive and costly, variation for agronomic traits in crop species is useful for generating regionally-adapted cultivars (Seiler 1992, Asselin et al. 2018) and is a source for novel alleles that could prove invaluable in breeding programs (Gur and Zamir 2004). Indeed, relatives of many crop species are reservoirs for pest and disease resistance traits, as wild populations are more often subjected to intense insect pressures than cultivated populations in managed environments (Zhang et al. 2017). To make use of variation in wild germplasm, researchers need (A) to have a methodology to test insect performance on the crop in a systematic way, and (B) to demonstrate that larval performance of insect herbivores differs in a measurable way when hosted by different crop genotypes. Although 'larval performance'—i.e., the effect of plant genotype or tissue on larval performance—does not directly translate to plant defenses (Agrawal 2011), a high throughput larval performance assay can identify genotypes that compromise larval growth can be a component of a multifaceted approach to identify well defended plant genotypes. In addition, variation for larval

performance is a valuable plant phenotype that can be selected for or be used to track germplasm for evidence of indirect selection (e.g., against secondary compound production) throughout the domestication process.

Silphium integrifolium (Michaux) (hereafter referred to as silflower) is a native herbaceous prairie species. Silflower is being domesticated for use as a perennial oilseed and forage (Van Tassel et al. 2017, Vilela et al. 2018). Perennial crops have deeper, denser, and more permanent roots than annuals and cover the soil with foliage for longer parts of the year (Kantar et al. 2016). Perennial crops can mitigate erosion in at-risk areas and have proven to be drought resistant in areas with inconsistent rainfall (Crews 2005, Culman et al. 2013, Crews and Rumsey 2017, Jungers et al. 2019). Silflower provides an additional ecosystem service as its abundance of flowers serve as a pollinator resource (Prasifka et al. 2017). Domestication of silflower has largely taken place in the central United States, with the majority of research on the developing crop occurring in Salina, KS at The Land Institute (Vilela et al. 2018) and at the University of Minnesota (Schiffner et al. 2020). Throughout silflower's nearly 20-yr domestication, major insect pests have been observed on silflower and are increasingly diminishing the efficacy of domestication (Prasifka et al. 2017).

The majority of the insect damage is occurring within the native range of silflower, central Kansas, and is caused by the native invertebrate *Eucosma giganteana* (Riley) (Lepidoptera: Tortricidae). Caterpillars of *E. giganteana* caterpillars, which are specialist feeders on *Silphium* spp., can disrupt breeding cycles and cause a reduction in seed production by consuming floral tissue and root crowns (Vilela et al. 2018). However, the plant is also commonly fed on by less specialized or even generalist pests. Stems have been observed to harbor the ironweed weevil *Rhodobaenus tredecimpunctatus* (Illiger) (Coleoptera: Curculionidae) (Prasifka et al. 2017), flower heads can be damaged by the sunflower headclipping weevil *Haplorhynchites aeneus* (Boheman) (Coleoptera: Curculionidae) (Hamilton 1974) and occasionally the sunflower moth *Homoeosoma electellum* (Hulst) (Lepidoptera: Pyralidae) (Prasifka et al. 2017). Leaves can be significantly damaged by the yellowstriped armyworm *Spodoptera ornithogalli* (Guenée) (Lepidopera: Noctuidae) (Prasifka et al. 2017), and the goldenrod leafminer beetle *Microrhopala vittata* (F.) (Coleoptera: Chrysomelidae, pers. obs.) (Hendrickson 1930, Johnson et al. 2019). Our growing understanding of the pest-plant interactions in silflower has prompted the rapid development and deployment of pest mitigation and prevention strategies based on practices developed for other crops, including parasitic biological controls (Lacey and Georgis 2012) and habitat management to enable natural enemies (Fiedler et al. 2008), or silphium-specific protocols such as trimming or mowing (Vilela et al. 2020). We believe, however, that there is also an abundance of untapped host plant resistance in this perennial species. Regarding silflower and its close relatives, Raduski et al. (2021) found considerable genetic variation within and between wild *Silphium* populations, and Stephan et al. (2018) showed that these populations differ phenotypically across environmental gradients. Gansberger et al. (2015) reported similar results for the silflower relative, cup plant (*Silphium perfoliatum* (*L.*)). Genetically divergent wild populations have been shown to harbor useful traits in other Asteraceae crops such as the common sunflower *Helianthus annuus* (L.) (Seiler et al. 2017). Here we evaluate diversity for traits related to herbivore weight gain in wild populations of silflower.

We developed a laboratory bioassay using the fall armyworm *Spodoptera frugiperda* (J. E. Smith) to test generalist insect larval performance on the leaves and flower heads of multiple silflower genotypes, one *S. perfoliatum* genotype, and one *H. annuus* line (HA89). Although fall armyworm is not currently a pest of silflower, it is closely related to the yellowstriped armyworm which does feed on silflower (Prasifka et al. 2017). Additionally, fall armyworm is a generalist whose host plants span 76 families including Asteraceae (Montezano et al. 2018) and the life cycle and development of fall armyworm are well-studied (Johnson 1987, Sparks 1979). Fall armyworm is commonly used in biological assays to determine the efficacy of pesticides, biological controls, and plant resistance (Jamjanya et al. 1990, Marenco et al. 1992, All et al. 1996, Davis et al. 1999, Meagher et al. 2004, Hardke et al. 2011, de Oliveira et al. 2016). These factors make fall armyworm a suitable and easily acquired candidate to determine whether there are differences in tissue palatability between silflower genotypes.

Methods

Plant Information

In 2020 we conducted two biological assays, one on the leaves and the other on the flower heads of eleven silflower genotypes, one cup plant genotype and the common sunflower inbred line HA89. Common sunflower was used as a control rather than maize because it is more closely related to silflower, thus presumably having a more similar phytochemical, nutritional, and physical composition to silflower than maize; and because fall armyworm larvae show similar growth and survivorship when fed sunflower leaves versus maize leaves in laboratory conditions (Dias et al. 2016). Floral and leaf tissues were used as these structures are commonly damaged by pests in breeding plots and would demonstrate the resistance of two critical tissues needed for a successful crop: the flower heads whose seeds would be harvested for oil, and the leaves which provide photosynthetic resources. Larval growth on one tissue type may not predict larval growth on the other, but a genotype that produces low larval growth in both tissue types could indicate a promising candidate for resistance.

Of the eleven silflower genotypes and one cup plant genotype, all except one silflower genotype ('AdAstra') originated from wild plants whose seed was harvested in 2016 within *Silphium*'s native range from remnant prairies in four states (Fig. 1). 'AdAstra' is a vigorous and partially disease resistant genet identified from one of the improved lines developed at The Land Institute (from wild silflower collected in nearby central Kansas prairies) (Vilela et al. 2018). In 2017 the eleven wild genotypes were planted in a common garden owned by The Land Institute in Salina, Kansas. They remained there until the spring of 2019 when one plant of each of the eleven wild genotypes was uprooted and cloned by splitting its rhizome. Simultaneously, the rhizomatous crown of 'AdAstra' was removed from a nearby breeding plot, where it had been growing for 3 yr, and split to form clones. Due to the varying sizes of the rhizomes, the number of clones per genotype varied (Table 1). All clones were potted in large fabric pots (Smart Pot, High Caliper Growing Systems, Oklahoma City, OK) containing peat-based media and mycorrhizal fungus inoculant (Pro-Mix BX, Premier Tech Horticulture, Rivière-du-Loup, Quebec, Canada) and placed outside in a hoop house to overwinter. In December 2019, all clones were moved into the greenhouse.

The average temperature and relative humidity (RH) in the greenhouse during March and April were 24.61°C, 62.2 RH and 25.18°C, 60.67 RH respectively. The greenhouse was equipped with LED lights set to turn on between the 6th and 16th hour if the irradiance level was below 150 W/m^2 for ten minutes and turn off if it was

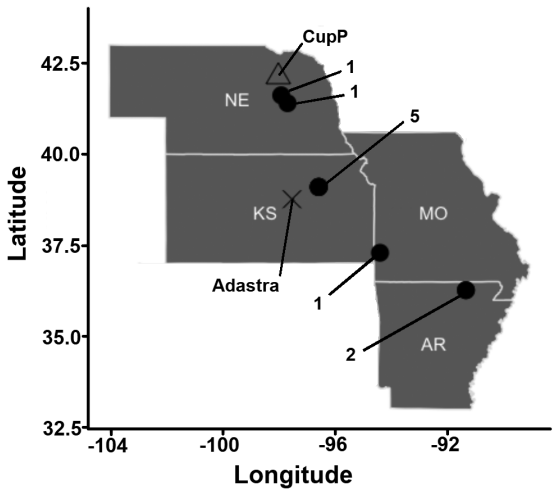

Fig. 1. Collection locations of the *Silphium* genotypes used in this study. Each black circle represents a prairie remnant where collection of seeds occurred in 2016 and is labeled with the number of genotypes from that prairie remnant used in the bioassay.

at or above 200 W/m^2 for ten minutes. This setup allowed the plants to receive at least 10 hr of light per day. Pots were sub-irrigated three times weekly using automatic watering benches and fertilized once with four tablets of the controlled release fertilizer Osmocote Plus 15-8-11 the day they were placed in the greenhouse. The Osmocote Plus 15-8-11 we used was supplied by Hummert International (Earth City, MO) and manufactured by ICL (Israel Chemicals, Ltd., Tel Aviv, Israel).

Insect Information

All fall armyworm larvae were obtained from Frontier Agriculture Services (Newark, DE), item L9179. According to the supplier, the fall armyworm colony originated from individuals collected from maize fields. Before the bioassay, fall armyworm larvae were reared and shipped on Frontier General Purpose Lepidoptera Insect Diet (F9772). Larva was generally used the same day they were received. At most, they were used a day later in which case they were kept in the dark between 70–75°C in their original container. According to information provided by Frontier the larvae for the leaf biological assay were likely 2nd instars. For the flower biological assay, the larvae were mostly 2nd instars, and a few were 3rd instars due to the 1-d delay in one case between the larva shipment and the beginning of the block of flower assays.

Equipment and Setup

All biological assays were performed in an environmental chamber (Percival Scientific, model I36VL) maintaining a temperature of 25 ± 1°C, Light-dark cycle of 14:10 and RH of 75 ± 10%. Whole leaves and whole flowers were cut from the plants in the greenhouse and placed on top of agar in an 85 × 12 mm plastic petri dish. Leaves often had to be halved, and layered to fit in the petri dishes and were sterilized with a sterilizing solution of 2 ml 95% denatured alcohol (ETOH) and 20 ml household bleach containing 7.5% hypochlorite per 170 ml tap water (Nowierski et al. 1995). Flowers were not sterilized because of their tendency to fragment when placed in a sterilization solution. The agar solution consisted of 7 g of agar, and 0.125 g of benzimidazole per 1 liter tap water (Nowierski et al. 1995). Our methods differed from Nowierski et al. (1995) with the substitution of tap water for distilled water.

Leaf Biological Assay

Preparations to start the leaf assay took place between 7–10 March 2020. At the time only genotype AR2 was flowering. The remaining genotypes and HA89 were bolting but not yet reproductive. All the caterpillars used for this assay were received from a single shipment on 10 March 10. On March 7 fifty agar plates were poured for each silflower genotype, cup plant genotype, and the HA89 sunflower line.

On March 9, fifty leaves were collected from each silflower genet, cup plant, and HA89. Clones of each genet were sampled as equally as possible. All leaves were harvested in the morning within one hour from the middle third of the stems. Leaves were sterilized by placing them in the sterilization solution, described above, for 3–5 min. Multiple batches of solution were necessary to sterilize all the leaves so sterilization solutions were used twice before being replaced. While not being sterilized, leaves were stored in a zip lock bag at 7.22°C. After sterilization leaves were placed on paper towels where excess water was removed, then while still wet they were cut with sterilized scissors to approximately fit the bottom of the petri dishes and placed on the agar. Each petri dish had at least two layers of leaf tissue. The lids were left off until the surfaces of the leaves were completely dry after which they were placed in the environmental chamber in random order (random.org). The amount of tissue per petri dish varied slightly but no caterpillar ran out of food.

On March 10, a single fall armyworm larva was weighed and placed on each plate. The process took six hours. After four days of feeding, fall armyworm larvae weight were again measured per caterpillar and the larvae were placed in ethanol for termination. We chose to stop feeding at four days for two reasons 1) a preliminary study with *Silphium* leaves embedded in agar resulted in nearly all the caterpillars in the treatment dying after 4 d, this was not seen in the control which also contained agar and 2) according to the incubation chart of Frontier, at our temperature and on adequate diet, larvae should be around the 4th or 5th instar after four days. As our goal was to see if there were differences between the silphium genotypes we opted to stop feeding at 4 d in case all the caterpillars in the treatments died or the caterpillars in the control pupated. We used OHAUS Explorer EX225D and EX124 balances for all weight measurements.

Data were analyzed using the Welch one-way Analysis of Variance (ANOVA) for unequal variance using the 'FSA' package (Ogle et al. 2021) in the statistical software R (R Core Team 2021). Proportional mass gain was set as the response variable and genotype as the explanatory variable. Proportional mass gain was calculated per caterpillar using the following formula:*(final mass – initial mass)/initial mass*. The data were square root plus one transformed to meet the assumption of normality. The Games-Howell post hoc test was performed using the 'userfriendlyscience' package (Peters 2017). *P*-values were adjusted using the Games-Howell method. Three influential points were removed before the analysis because according to the function outlierTest from the car package (Fox and Weisberg 2020) and a qqplot they shifted the mean significantly. With the influential points in the analysis there are thirty-two significant contrasts, with the influential points removed there are thirty-five significant contrasts (Supp Tables 1A and 2A [online only]). Standard model validation procedures were performed. During the experiment, some replicates contained caterpillars that died (no more than 4 replicates per genotype, Table 1). These replicates were excluded from the analyses since weight gain was not determined for them.

Flower Head Biological Assay

Because different genotypes flowered at different times the flower head biological assay was performed in five blocks using an

Table 1. Number of clones and replicates for the 12 *Silphium* spp. genotypes plus the variety of annual sunflower

Genotype	Clones	Flower assay replicates	Leaf assay replicates	Flower assay replicates prepared
AR1	1	45	50	47
AR2	6	66	50	70
KS1	5	49	50	51
KS2	5	50	49	50
KS3	5	48	49	52
KS4	4	52	49	56
KS5	5	48	49	50
MO1	3	50	49	50
CupP	9	50	49	50
NE1	5	49	48	50
NE2	3	45	49	48
'AdAstra'	7	68	50	70
HA89	20	49	46	50

Note that for the flower assay only 4 clones of HA89 plants were used because individual flower heads were very large. For the leaf biological assay 50 replicates for each genotype were prepared. The difference between 'replicates prepared' and 'replicates' is due to fall armyworm mortality.

unbalanced design. This means that in each block there was an uneven number of replicates per genotype. The maximum number of replicates per genotype per block was twenty and the minimum five. Except for HA89 which was only present in three blocks, all other genotypes were present in all the blocks. The original number of replicates per silflower genotype, cup plant genotype, and HA89 ranged between 50–70 (Table 1). The flower head weight of each silflower genotype, cup plant, and HA89 was determined by measuring three mature flower heads as the plants began to bloom. These weights were averaged to provide the flower head weight per genotype. A mature flower head was defined as having a fully open set of ray florets (petals) and at least one ring of open male florets in the central disc (revealing anthers). Approximately 4 g of flower head tissue—more tissue than could be consumed within 4 d (based on preliminary trials)—was provided to each fall armyworm larvae. All flower heads were used the same day they were harvested.

For each block, agar was poured, the flower heads were harvested, placed on the agar and fall armyworm larvae were weighed and placed one per plate in the span of two days. The agar solution used was the same as that used in the leaf bioassay. Plates were then randomized and placed in the environmental chamber. After four days, each fall armyworm larva weight was measured again and the experiment terminated as in the leaf assay.

Data were analyzed with a mixed effect model fitted by Restricted Maximum Likelihood using the 'nlme' package (Pinheiro et al. 2021) in R. The model is a random intercept model with the variable 'Block' set as a random effect, proportional mass gain (see formula in the leaf section) as the response variable and genotype as the explanatory variable. Weights were added to the model to meet the assumption of heteroscedasticity. Standard model validations were performed, and a type III ANOVA was performed on the model. Pairwise comparisons with Tukey adjusted *P*-values were performed using the 'emmeans' package (Lenth 2021). Here, we use a mixed model instead of an ANOVA as in the leaf data analysis, because here, unlike in the leaf bioassay, it was necessary to include block effect. Dead caterpillars (no more than 4 per genotype, Table 1) were excluded from the analysis since a final mass was not measured.

Regression

To determine whether there was a correlation between fall armyworm performance on leaves and flower heads we performed a regression. The average proportional weight gain for each silphium genotype and HA89 was calculated for the leaf data and the flower head data. Therefore, we had a sample size of 13 for our regression analysis. In our model, flower head proportional weight gain was set as the response variable and leaf proportional weight gain as the explanatory variable. The simple linear regression assumptions of normality and heteroskedasticity were not met, therefore we analyzed the data using the nonparametric Thiel-Sen Estimator/regression. Because HA89 was a clear outlier, we performed the analysis with and without HA89. The R package 'wrs' by R.R. Wilcox was used to perform this analysis (Wilcox 2020).

Resampling

Time and resources are always a limiting factor; therefore, to expedite screening of plant germplasm, it is important to know the minimum number of replicates needed in this bioassay to identify a plant accession with exceptionally high or low insect herbivore susceptibility. To that end we performed a resampling analysis using the 'replicate' and 'sample' functions in base R to simulate the effect of reduced laboratory replication on our ability to detect differences between 'reference genotypes,' (HA89, 'AdAstra') on which fall armyworm gained the most (with full replication of about 50; Table 3) and each of several genotypes on which larvae gained less weight ('test genotypes': 'AdAstra', AR1, AR2, and KS3). 'AdAstra' was included in both groups, having a higher gain than the other *S. integrifolium* genotypes but a lower gain than HA89. Cup plant was statistically indistinguishable from 'AdAstra' and was included with the test genotypes group as a control. For the leaf assay, the individual caterpillar proportional weight gain data were resampled with replacement. Random draws of data from pairs of plant genotypes were made and the means for each plant were compared 10,000 times for each simulation. The simulated sample replication levels were 30, 20,10, and 5. The proportion of instances that the mean of the test genotypes was equal to or exceeded that of one of two reference genotypes was calculated. The same was done for the flower head biological assay.

Results

Leaf Biological Assay

Proportional weight gain of fall armyworm larvae on leaves differed by plant species and genotypes ($F_{242.19}^{12} = 26.452$, $P < 0.001$). Mean fall armyworm proportional weight gain was significantly lower on cup plant and all silflower genotypes than on annual sunflower ($P < 0.001$, Fig. 2). Among silflower genotypes the results varied;

AR1 and AR2 produced significantly lower proportional weight gain than seven of the silflower genotypes, while cup plant only had significantly higher proportional weight gain than AR2 (Fig. 2). Larvae placed on 'AdAstra' leaves had proportional weight gain that was statistically indistinguishable from all but three of the wild silflower genotypes (Fig. 2, see Supp Table 1A [online only]).

Flower Biological Assay

Fall armyworm proportional weight gain in larvae that were fed flower heads also differed by genotype and species (Type III ANOVA $X_{12}^2 = 387.39$, $P < 0.001$). All *Silphium* genotypes (wild silflower, 'AdAstra', and CupP) produced significantly lower proportional weight gain in fall armyworm larvae than the HA89 sunflower. Among the silflower genotypes, larvae that consumed 'AdAstra' (the most domesticated genotype of silflower) had significantly greater proportional weight gain than six of the wild genotypes (Fig. 2, Supp Table 3A [online only]).

While AR1 and AR2 performed similarly between both leaf and flower assays this was not the case for all genotypes. In the leaf assay KS2 was significantly different than AR1 and AR2 but, for the flower head assay there were no differences. In the flower head assay, MO1 was significantly different than seven silflower genotypes while for the leaf assay MO1 significantly differed from eight. For more differences between the flower and leaf assay results, see Fig. 2.

Regression

The nonparametric Theil-sen regression was performed between flower head and leaf assay results to determine whether leaf assay results could predict flower assay results. In both the analyses with HA89 and without we found no significant predictive power between leaf assay results and flower assay results (Table 2, Fig. 3).

Resampling Analysis

As expected, resampling the data from pairs of genotypes not found to be significantly different in caterpillar performance in the full bioassay frequently gave a high rate of incorrect means rankings (see unshaded cells in Table 3). Rankings are considered incorrect if they are different than the rankings shown by letters in Fig. 2, which are based on the full set of about 50 replicates. Comparing all pairs of plant genotypes previously (Fig. 2) found to be statistically significantly different (see shaded cells in Table 3), the means were correctly ranked at least 95 percent of the time with only 20 (simulated) replicates and for all comparisons between sunflower and silflower, with only 5 replicates required to achieve at least 95% correct ranking. With 10–30 replicates, incorrect ranking was never seen in 10,000 simulations. The optimum number of replicates will depend on the goals of the bioassay and the experimenter's tolerance of false positives or negatives. However, based on this analysis it appears that more than 20 replicates would almost never be required. For rapid assays for extreme outliers, such as silflower

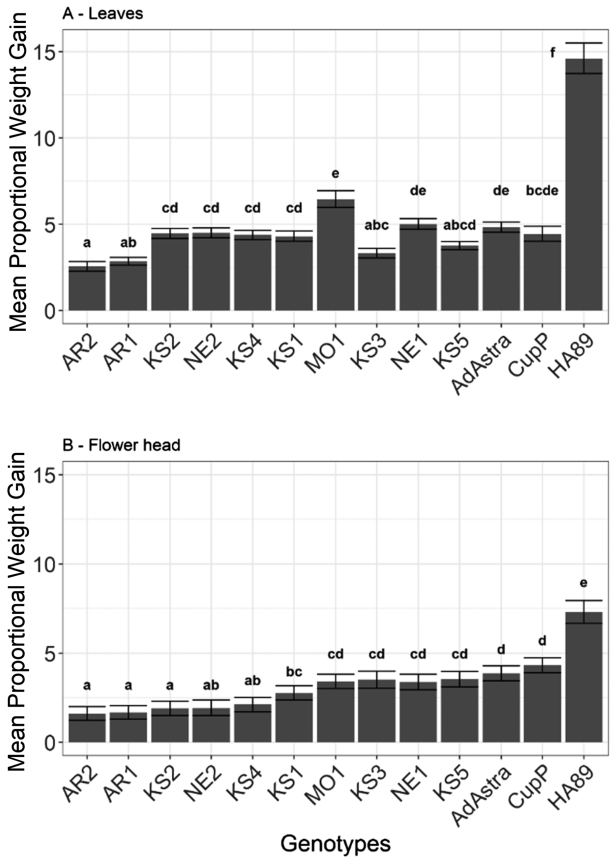

Fig. 2. Bar plot with standard error bars. Groups that share a letter are not significantly different at the 0.05 level. (A)The leaf effect of the different plant groups on fall armyworm proportional weight gain; the means and standard errors were back-transformed from the square root +1 transformation (B) Same as A but with flower heads instead of leaves and the means and standard errors did not need to be back-transformed. Note that for both panels the mean displayed is the marginal mean not the Arithmetic mean. 'AdAstra' is the most improved *Silphium integrifolium* line, CupP is of the species *S. perfoliatum*, and HA89 is a sunflower inbred line (*Helianthus annuus*).

genotypes that have lost resistance traits and produce a feeding response similar to domestic sunflower (e.g., HA89), 5 replicates could safely be used, a 10-fold reduction from our initial protocol of 50 replicates per plant genotype.

Flower head tissue assays incorrectly ranked the lowest caterpillar performance silflower genotypes (wild accessions AR1 and AR2) and the highest-performance individual (semi-domesticated 'AdAstra') less frequently than leaf tissue assays, with fewer than 1% wrong ranking versus about 5% for five-replicate simulations (flower heads and leaves respectively, Table 3). On the other hand, the mis-ranking rate was slightly higher for comparisons between all *Silphium* genotypes and sunflower when flower heads were assayed instead of leaves. This result suggests that heads and leaves have similar effects on larval performance but are not completely interchangeable for the purpose of screening germplasm.

Discussion

Our novel bioassay uncovered that weight gain of fall armyworm differed significantly between *Silphium* (silflower and cup plant) genotypes, and that fall armyworm gained substantially less weight on *Silphium* plants than an inbred annual sunflower genotype. Fall armyworm larvae gained particularly little weight on both the flower and leaves of the wild collected silflower genotypes AR1, and AR2. Multi-year field observations are required to verify that these genets offer a reduction in herbivory on important tissue types compared to other *Silphium* accessions. The cause of the difference in potential resistance or tolerance between genotypes and species is still unknown. We suspect forage quality, physical defenses, such as trichomes, or chemical compounds such as resins and secondary metabolites may play a role in defense (Mason et al. 2016) although, a combination of these defense strategies is likely (Agrawal 2011). Further study of the traits unique to the individuals resistant to fall armyworm herbivory will clarify some mechanisms of defense important for silflower resistance.

Additionally, the nonsignificant correlation between caterpillar feeding on leaf tissue and floral tissue suggests that fall armyworm feeding on one type of tissue does not predict feeding on another tissue on the same plant. The variability in results depending on genotype and tissue type highlights the need to be intentional in selecting which tissue type to test when using assays to study caterpillar performance in future studies. This variability may be a result of differences in the pests that have historically damaged these tissue types as well as a difference in overall herbivory pressure in geographically distinct wild populations. Pests that feed on silflower leaf tissue (leaf miners, caterpillars) are not necessarily the same pests that feed on the floral tissue (seed and capitulum burrowers) (Prasifka et al. 2017). In addition, herbivory pressure across the native range of *Silphium* species is also likely diverse, which could result in a diversity of defense strategies (Atsatt and O'Dowd 1976).

The next step towards using detached plant structures (leaves, flower heads) in laboratory insect bioassays as a tool for comparing plant genotypes in the silflower germplasm enhancement program will be to validate that genotypes that produce poor caterpillar growth in vitro also experience reduced generalist insect herbivory under field conditions. While bioassays requiring large numbers of replicates are impractical for use in crop improvement because of the labor required, inadequate growth chamber space, and inability of un-cloned plants to produce sufficient numbers of flower heads or leaves, we have shown here that fewer than five flower head bioassay replicates or 10 leaf replicates enable several of the most interesting wild silflower germplasm in this study to be resolved from the 'elite' reference genotype. This is relevant for germplasm exploration: plant geneticists need to be able to screen large collections of wild germplasm to identify accessions or individuals with exceptional qualities that would make them good candidates as donors of rare genetic variation for the improvement of elite populations.

Although both leaf and flower head bioassays seem feasible for identifying rare accessions with better herbivory resistance traits than the current elite lines, going forward leaf bioassays are preferred because they can be done on first-year plants (silflower does not generally flower until the second year), so leaves are much easier to produce than flower heads under controlled conditions. According to our resampling simulations, screening 10 arbitrarily chosen wild accessions with 5 replicates each would have allowed us to correctly identify a genotype such as AR2 as a potential gene donor 95.6% of the time. Presumably, screening 100 wild accessions with this level of replication would have been feasible (the same total number of replicates as screening 10 accessions with 50 reps each) and would have had a nearly 100% chance of identifying plant genotypes significantly better than the reference 'elite genotype', especially as the odds of

Table 2. Results of the Theil-sen regression

Analysis	Intercept	Slope	SE	*P*-value
With HA89	0.6603	0.4522	0.2861	0.0701
Without HA89	0.7201	0.4399	0.3928	0.1870

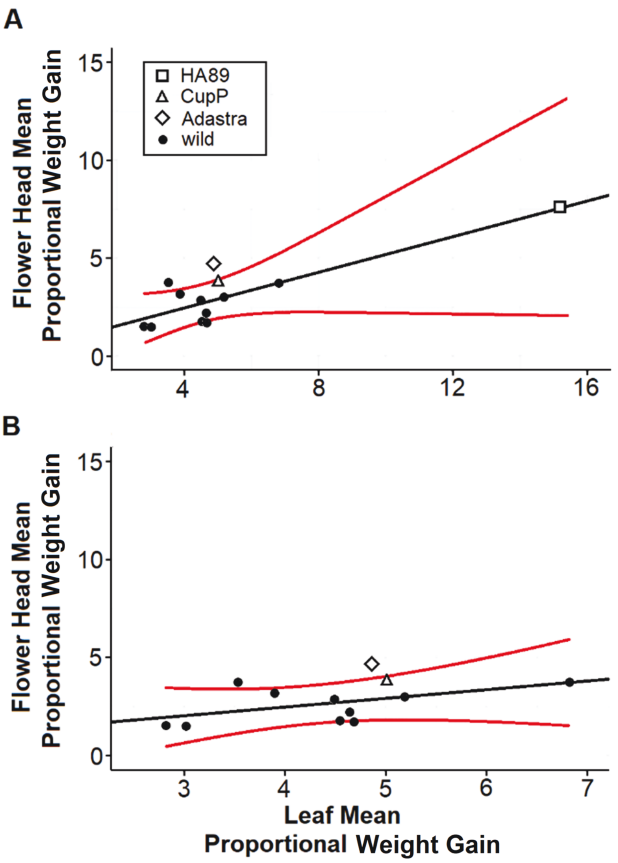

Fig. 3. Theil-sen regressions for the leaf and flower head biological assay results. A) with HA89, B) without HA89. In each graph, slope and CIs are shown.

Table 3. Results of the resampling analysis for the leaf and flower head simulating experiments with fewer independent caterpillar replicates

Test genotype	Simulated sample size (number of replicate caterpillars)	Proportion of 10,000 means comparisons with reference genotypes (AdA, HA89) when test genotype means were equal or higher.			
		Leaf tissue assay		Inflorescence tissue assay	
		AdA*	HA89	AdA*	HA89
AR1	30	0.000048	0	0	0
AR1	20	0.000733	0	0	0
AR1	10	0.012158	0	0.000097	0
AR1	5	0.054225	0.000001	0.004546	0.000043
AR2	30	0.000013	0	0	0
AR2	20	0.000307	0	0.000002	0
AR2	10	0.007776	0	0.000299	0
AR2	5	0.044048	0.000002	0.008361	0.000072
KS3	30	0.003693	0	0.477527	0
KS3	20	0.014395	0	0.482537	0.00005
KS3	10	0.060043	0	0.48993	0.003361
KS3	5	0.13416	0.00002	0.494819	0.027969
CupP	30	0.410271	0	0.959261	0.000091
CupP	20	0.426266	0	0.923607	0.001091
CupP	10	0.446274	0	0.846187	0.015967
CupP	5	0.460479	0.000347	0.77001	0.067534
AdA*	30	na	0	na	0.000002
AdA	20	na	0	na	0.00008
AdA	10	na	0	na	0.004032
AdA	5	na	0.000186	na	0.031866

Shading reflects the post hoc means comparison for the full bioassay (approximately 50 reps, Fig. 2). Lightly shaded cells: test genotype mean proportional fall armyworm weight gain significantly lower than reference genotype. No shading: test genotype means lower than reference genotypes but not significantly. Darkly shaded cells: test genotype mean higher than reference but not significant. *'AdAstra' is abbreviated AdA.

encountering individuals with even more extreme phenotypes than AR2 would be greater with a larger sampling of the wild germplasm.

It is important to note that this bioassay demonstrates only differences in larval performance with a generalist herbivore. In future research, specialist pests known to target silflower leaf and floral tissue should also be tested to determine whether or not they show a similar response. Insect specialists such as *Eucosma giganteana* have proved extremely difficult to rear in laboratory settings (Chérémond, personal communication 2020). Understanding of specialist insect pest rearing would enable breeders to incorporate specialist pest in the bioassay protocol to measure weight gain of known pests of silflower genotypes. Although fall armyworm use enables efficient screening, separate testing with specialist herbivores could prove essential to developing resistant silflower breeding lines.

To our knowledge, insect bioassays have not previously been used to screen wild silflower germplasm for the benefit of crop breeders, but the results presented here suggest that such an approach would be feasible. The study described here could be adapted in future studies to screen silflower resistance to different pests, as well as investigate the response of other parameters of pest herbivore development. Differences in fall armyworm weight gain on leaf and floral tissue of diverse *Silphium* genotypes in this study demonstrate a quick, replicable method to screen germplasm for genotypes less favorable to a generalist pest.

Supplementary Data

Supplementary data are available at *Environmental Entomology* online.

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