Response to Kozlov *et al.*: Inaccurate estimation of biases in herbarium specimen data

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Summary. Kozlov and colleagues[1] call into question the application of herbarium specimens to quantify historical patterns of herbivory[2–5]. It is already widely appreciated that collectors of herbarium specimens may tend to avoid insect damage, thus making herbivory estimates from herbarium specimens potentially down-biased[2]. However, Kozlov *et al.* additionally suggest that variation in sampling selectivity among collectors may misrepresent patterns of herbivory in nature. The authors sought to quantify these biases by collecting and contrasting insect herbivory data across 17 plant species from herbarium versus standard field ecological sampling procedures. They concluded that herbivory estimates from herbarium specimens are highly variable, rendering them an inaccurate representation of herbivory in nature. Our re-analysis of Kozlov *et al*.'s data, in contrast, reveals that herbarium specimens indeed provide a useful record of these symbioses. We demonstrate that Kozlov *et al*. were misled by analyzing comparisons derived from very few samples, which provide highly noisy estimates.

Main text. Kozlov *et al.* [1] assert that estimates of herbivory damage derived from herbarium specimens are unreliable. Assessing leaf area removed by insects to infer herbivory is a common practice[6]. However, it is well established that insect damage is distributed unevenly; some plants experience extensive damage, whilst others exhibit little or no signs of herbivory (e.g.[7]). It is critical, therefore, to sample multiple individuals when estimating species means, especially when assessing single plant species from a given locality (e.g.[8,9]). In their study, Kozlov *et al.* gathered too-few specimens to infer reliable estimates of herbivory (n=1 to n=10 for ecological samples, and n=3 to n=20 for herbarium samples). The pathologies resulting from such under-sampling are evident in Fig. 1 presented by Kozlov *et al*. Herbivory estimates derived from ecological versus herbarium sampling vary widely when inferred from only a few herbarium

samples, but converge when sample sizes are higher and comparable between sampling strategies. This trend is clearly observable when species are sorted by total sample size (Fig. 1a). For example, herbivory estimates for *Betula pubescens* and *Rubus idaeus*, the only focal species in which sample size is high for both categories, align closely (Fig. 1a).

Using Kozlov *et al*.'s published data, we show that herbivory estimated from ecological and herbarium specimens converge exponentially with sample size (Fig 1b; Fig. S1). By recreating Fig. 3 from Kozlov *et al*., we also demonstrate that herbivory estimates from these two sampling methodologies exhibit a broadly linear relationship which is disrupted by a few outliers characterized by small sample sizes (Fig. 2). If we restrict their data to include only species for which n=10—still a small sample size for noisy ecological data—the relationship between these metrics is linear and highly significant (Fig. 2, p= 0.014, r^2 = 0.39). Therefore, while Kozlov *et al.*'s data provide evidence that estimates of herbivory on herbarium specimens are down-biased, as suggested previously[2], differences between species' estimates are highly consistent when sampling effort is sufficient, contrary to the author's published conclusion. Further, we argue that residual species differences in herbivory estimates inferred from herbarium specimens does not negate their utility for investigations of global change response. For example, in a comparison of seasonal flowering sensitivity to temperature between observational and herbarium data, Davis *et al.*[10] demonstrated that while species-level phenological sensitivity measurements from herbarium specimens did not predict phenological sensitivity measured in the field, the effects of seasonal temperature on flowering within species still aligned among field and herbarium datasets.

In an additional analysis, Kozlov *et al*. mistakenly suggest that herbarium specimens cannot provide reliable estimates of herbivory in nature because preferences for damaged specimens vary among herbarium curators. We agree that curator specimen preferences are likely to vary, but disagree with the conclusion that this necessarily reduces the utility of herbarium specimens. Differential biases among collectors should not affect analyses unless collector bias varies systematically across axes of interest. Potential variation in cultural practices among herbaria suggests that we should exercise caution when comparing herbivory estimates derived from herbaria among regions. We otherwise see no reason why collector bias would vary systematically across pertinent axes of interest, such as temperature, where collector preferences would simply add noise, and biases may be addressed statistically, for example, by including curator as a random effect in models (see[2]). Nonetheless, we recognize that added noise could be problematic in statistical model fitting given the large natural variability in herbivory damage[11]. New machine learning methods to rapidly quantify herbivory from a large number of specimens[12] provide promise for the future by helping decrease the noise-to-signal ratio in datasets.

Conclusion: While biases in herbarium data are well recognized[13,14], there remains tremendous value in these historical specimens[3,15]. Our re-analysis of Kozlov *et al*.'s data reveals that herbarium specimens provide a useful record of historical herbivory, contrary to the authors published conclusions. We fully support efforts to validate herbivory estimates derived from herbarium samples with gold standard ecological sampling but urge the use of power analyses and/or rarefaction curves to ensure samples sizes sufficient to yield reliable estimates. We also note that while largescale efforts to better quantify collector bias in specimen data across species

are useful, we are often more interested in temporal and spatial trends within species to capture the fingerprint of global change (e.g.[16]). We hope that Kozlov *et al*.'s disproportionate criticism of herbarium specimens as a source of species interactions data will not discourage ecologists from leveraging the information captured within these specimens, which are increasingly being mobilized online and represent a rich resource for inferring past ecologies[17].

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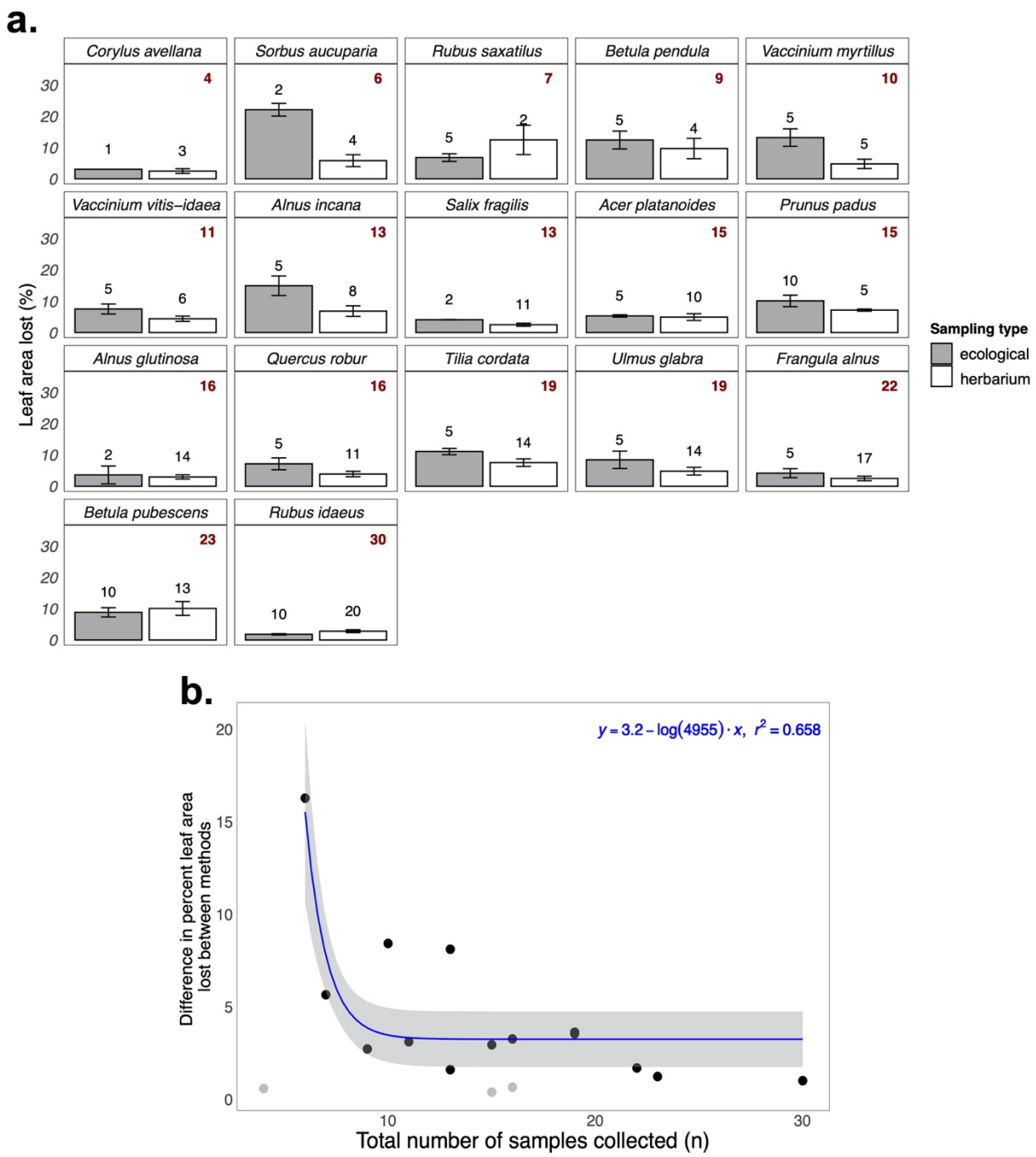


Figure 1. Effects of sample size on herbivory estimates. a) Raw data describing leaf area lost to insect herbivores detected using different sampling methods in Kozlov *et al*.[5,6]. Note that sample sizes differ slightly from those reported in Kozlov *et al*. because of a technical error that caused minor data misreporting in the original paper's supplement and in the main text figures. b)

Effects of total sample size (ecological + herbarium) on differences between herbivory estimates collected using ecological vs. herbarium sampling. The mean estimate from the fitted model is shown in blue (±SE in grey); model parameters are presented in the upper righthand corner ($F_{1,12}$= 25.97, p= 0.0002). The r^2 value reported is adjusted r^2. Values on the y-axis are the absolute differences in herbivory estimates between sampling strategies. Black symbols represent species for which leaf area removed shows notable divergence (>1% between sampling strategies). Grey symbols indicate species for which herbivory metrics converge across sampling metric, thus supporting the accuracy of herbivory data from herbarium specimens.

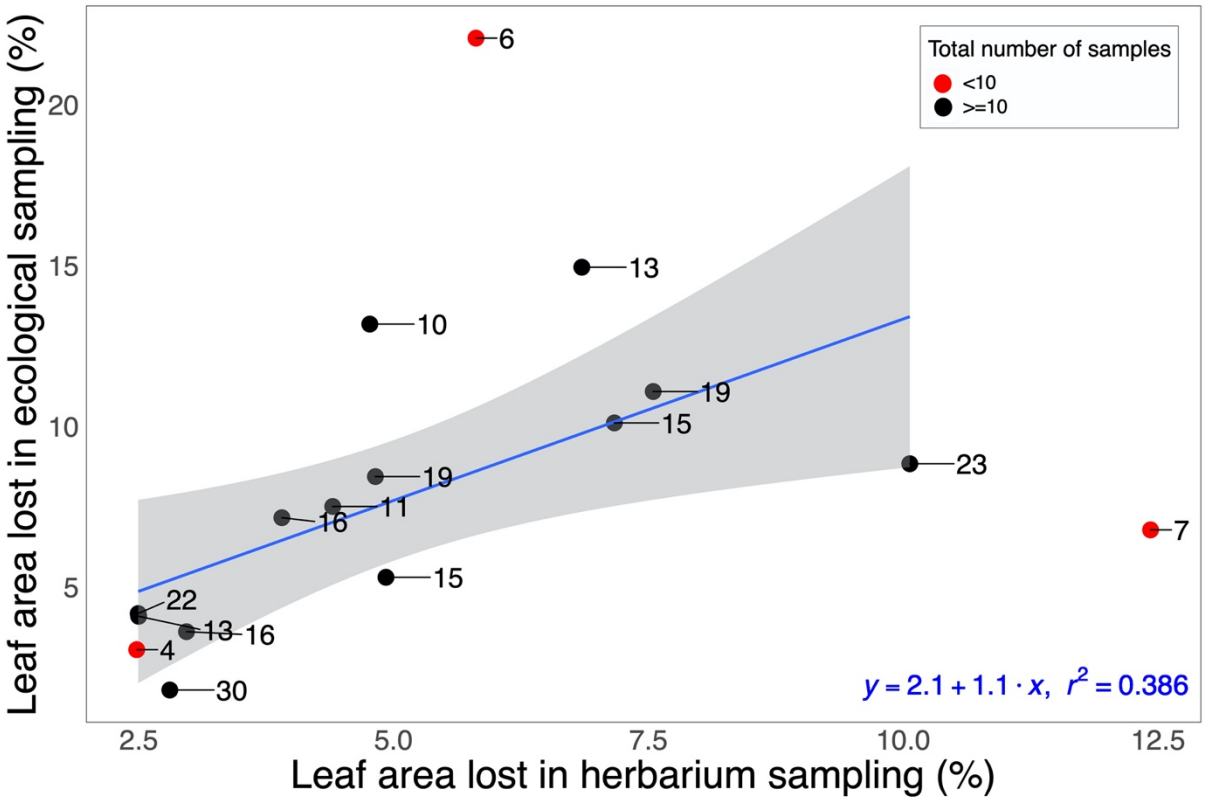
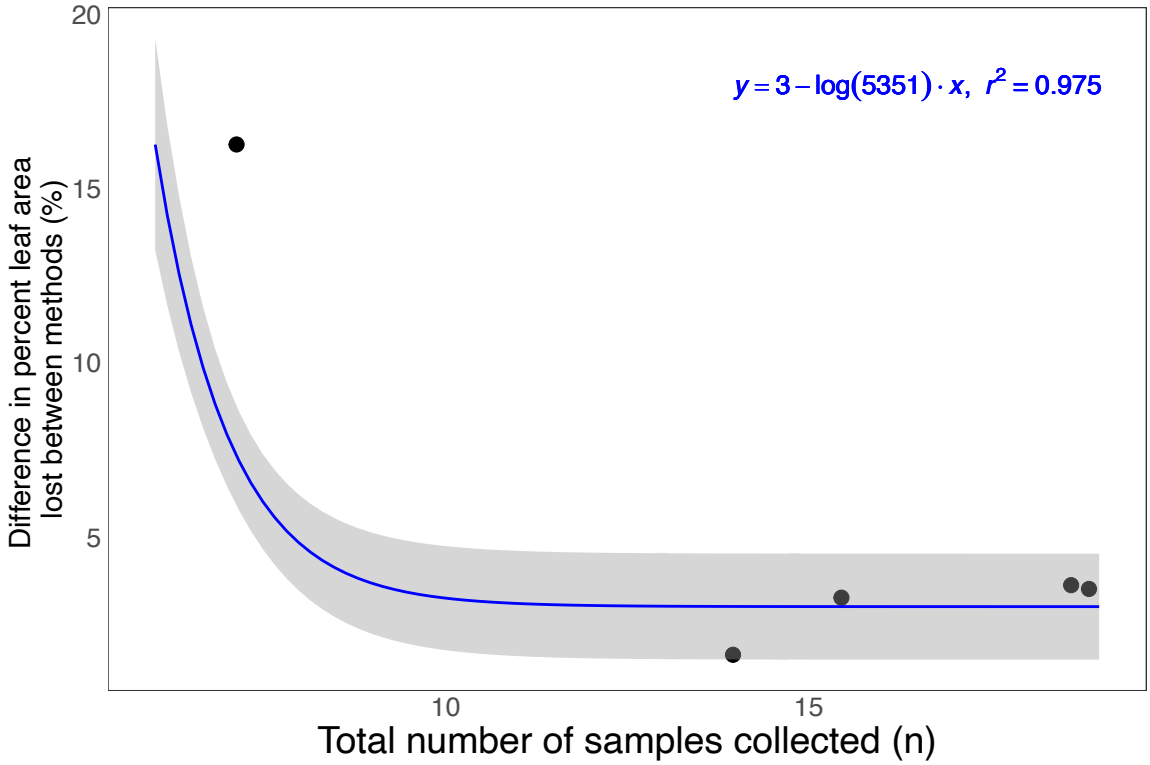


Figure 2. The relationship between herbivory estimated from ecological vs. herbarium specimens. Each data point represents a species, and numbers indicate total sample sizes (ecological + herbarium). The model fitted to species with >10 specimens collected (species in black) is shown in blue (±SE in grey). Associated model parameters are presented in the lower righthand corner ($F_{1,11}$= 8.56, p= 0.014). The r^2 value reported is adjusted r^2. Another model which included outliers for which <10 total specimens were collected (species in red) approached significance ($F_{1,15}$= 3.12, p= 0.098).

156 **Supplementary Information**



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159 **Figure S1.** Effects of total sample size (ecological + herbarium) on differences between
160 herbivory data collected using ecological vs. herbarium sampling. The subset of species plotted
161 here are those for which Kozlov et al. detected significant differences in herbivory estimates
162 from ecologically and botanically collected specimens. The model structure used to assess this
163 relationship and create the curve displayed here—mean in blue and ±SE in grey—are in the
164 upper righthand corner. The sample sizes in this analysis are low, and thus the parameter
165 estimates and significance values should be interpreted cautiously for this dataset ($F_{1,3}$= 154.00,
166 p=0.001). The r^2 value reported is adjusted r^2.
167