

Supplementary Material 2: Sampling method bias

The concern of sampling method bias and sensitivity across natural enemy taxa was considered in the South region where a diverse complex of parasitoids and predators had previously been detected when inspecting all leaves of a plant (1). The two-leaf sampling method was adopted for the large region comparative study because of its simplicity for the investigators contributing to the data set. It could be used in field without need of laboratory facilities. As noted in the results, the species complex detected was similar to previous reports including reports based on inspecting the whole plant (1). The large region study also estimated natural enemy abundance. Although the large region study focused on regional comparisons of natural enemy abundance and activity and not cross-taxa comparisons of abundance, low efficiency in sampling selected taxa may affect ability to make cross region comparisons.

Therefore, the two-leaf inspection method used in the large-region comparative study was compared to a more thorough whole plant destructive sampling method where plants were quickly bagged, cut, and taken to the laboratory for natural enemy recovery. This sampling bias investigation was conducted in 2019 at one of the study sites (Corpus Christi, TX), where both parasitoids and predators were readily observed in sorghum fields infested with *M. sorghi*. During sorghum vegetative growth, 80 plants (160 leaves) were randomly chosen using the two-leaf sampling method, and 16 plants (112 leaves) were randomly chosen using the whole plant destructive sampling method. Aphid natural enemies were separated by taxa and counted in field (two-leaf method) and in laboratory (whole plant destructive sampling method). The relative sensitivity of the two methods were compared using a two (method) by n (taxa) contingency table analysis (2). Extent of sampling effort was normalized by adjusting the natural enemy recoveries on a 100 leaf count basis.

There was no indication of sampling bias using the two-leaf sampling method in field compared with the more thorough laboratory inspection of the whole plant. The proportion of parasitoids and predators collected did not differ by the sampling method used ($\chi^2 = 3.41$, d.f. = 1, $P = 0.065$, $n = 1,191$ total number of natural enemies collected) in a two (method) by 2 (parasitoid and predator taxa) contingency table analysis (Table 1). Sampling bias was also not detected when separating the collected insects by the taxa used in the large region study used ($\chi^2 = 3.86$, d.f. = 3, $P = 0.28$) in an expanded two (method) by 4 (two parasitoids [*Aphelinus nigritus* and *Lysiphlebus testaceipes*], coccinellids, syrphids, and lacewings [chrysopids and hemerobiids]) contingency table analysis. Syrphids and lacewings were combined to meet the recommended contingency table criteria that $n > 5$ in all categories (Table 2). We concluded that the two-leaf sampling method was satisfactory for the large region comparative study.

Table 1. Results of the two (sampling method, rows) by two (natural enemy taxa, columns) contingency table analysis. Observed frequency of detection is followed by the expected frequency in parentheses based on the hypothesis of independence. Cell χ^2 values are placed in brackets. Total $\chi^2 = 3.41$, d.f. = 1, $P = 0.065$.

	Parasitoid	Predator	Row total
Two-leaf method	264 (257) [0.18]	12 (18.8) [2.44]	276
Whole plant method	846 (853) [0.05]	69 (62.2) [0.71]	915
Column total	1,110	81	1,191 (Grand total)

Table 2. Results of the two (sampling method, rows) by four (natural enemy taxa, columns) contingency table analysis. Observed frequency of detection is followed by the expected frequency in parentheses based on the hypothesis of independence. Cell χ^2 values are placed in brackets. Total $\chi^2 = 3.86$, d.f. = 3, $P = 0.28$.

	<i>Aphelinus nigrinus</i>	<i>Lysiphlebus testaceipes</i>	coccinellids	syrphids + lacewings	Row total
Two-leaf method	254 (246) [0.29]	10 (11.9) [0.29]	7 (10) [0.89]	5 (8.6) [1.50]	276
Whole plant method	805 (813) [0.09]	41 (39.2) [0.09]	37 (34) [0.27]	32 (28.4) [0.45]	915
Column total	1,059	51	44	37	1,191 (Grand total)

References

1. Maxson EL, Brewer MJ, Rooney WL, Woolley JB. Species composition and abundance of the natural enemies of sugarcane aphid, *Melanaphis sacchari* (Zehnter) (Hemiptera: Aphididae), on sorghum in Texas. *Proc. Entomol Soc Wash. Soc.* (2019) 121: 657-680. doi: 10.4289/0013-8797.121.4.657
2. Freund JE, Walpole RE. Mathematical statistics, 3rd ed. Prentice-Hall, Englewood Cliffs, NJ. (1980).