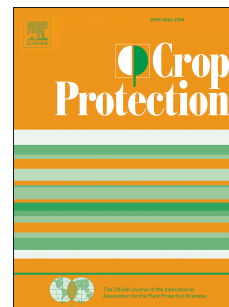


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Active and passive sampling methods for grubs of the Asiatic garden beetle, *Maladera formosae* (Coleoptera: Scarabaeidae), in a corn-soybean rotation

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12 **Active and passive sampling methods for grubs of the Asiatic garden beetle, *Maladera***

13 ***formosae* (Coleoptera: Scarabaeidae), in a corn-soybean rotation**

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19 Abstract

20 Beginning in the mid-2000s, grubs of the Asiatic garden beetle, *Maladera formosae*
21 (Brenske) (syn. *M. castanea* [Arrow]), emerged as significant early-season pests of field corn
22 grown in sandy soils of the Great Lakes region. Overwintered grubs move towards the soil
23 surface in springtime to resume feeding on roots, causing stand losses of up to 40%. Scouting for
24 *M. formosae* is problematic as the grubs are subterranean, and the adults are nocturnal. The
25 objective of this study was to evaluate sampling methods currently in use for other annual white
26 grub species specifically for use with *M. formosae* grubs in corn production systems. In a series
27 of two experiments, we evaluated the ability of the compact cutter, golf hole cup cutter, and
28 wire-mesh bait station to detect *M. formosae* grubs and sense varying population densities. The
29 cup cutter, which takes a deeper but smaller soil volume sample than the compact cutter,
30 sampled more grubs per soil volume and was more sensitive to smaller grub populations. The
31 bait station, a passive sampling technique, and cup cutter were both successful at detecting *M.*
32 *formosae* before planting. However, the cup cutter is more adequate and economical since it can
33 be used with less equipment in a single trip to the field. Identification of a standardized sampling
34 methods will enable researchers to predict future infestations and ultimately lead to the
35 development of economic threshold levels for *M. formosae* in row crop agroecosystems.

36 Keywords

37 Asiatic garden beetle, annual white grub, sampling methods, field crops, Scarabaeidae

38 **Highlights**

- 39 • Cup cutter is more sensitive to varying grub densities than compact cutter
- 40 • Bait station and cup cutter allow for passive pre-plant sampling of grubs in corn
- 41 • Cup cutter requires only one trip to the field and less supplies than bait station

42 **1. Introduction**

43 The Asiatic garden beetle, *Maladera formosae* (syn. *M. castanea* [Arrow, 1913])
44 (Coleoptera: Scarabaeidae: Melolonthinae) (Fig. 1), has been present in North America since
45 1921 (Hallock, 1929) and is historically a pest of turfgrass (Hallock, 1930), ornamentals
46 (Hallock, 1930), and vegetables (Hallock, 1934). However, the grubs have recently emerged as a
47 significant, annual, early-season pest of field corn (Fig. 2) in northern Indiana (Krupke et al.,
48 2007), southern Michigan (DiFonzo, 2007), and northern Ohio (Hammond, 2013). As with many
49 other annual white grub species (i.e., the entire life cycle is completed in one year), rising
50 springtime temperatures stimulate overwintered second and third instars (Fig. 1B) to move
51 towards the soil surface to resume feeding on roots until they pupate and mature into adults by
52 summer (Tashiro, 1987). The upward migration and resumption of feeding coincides with the
53 planting of corn and other field crops in the Great Lakes region. Grub feeding on fragile corn
54 seedling roots causes plants to stunt, wilt, discolor, and ultimately die (Fig. 2), and plant stand
55 losses in excess of 40% are not uncommon (Hammond, 2013).

56 It is difficult to scout for and distinguish white grub species since they occur in grub
57 complexes that are comprised of several species, are subterranean, physically similar to the
58 untrained eye, and cause similar feeding damage and symptoms (Potter, 1998; Vittum et al.,
59 1999; Reding and Klein, 2007; Shetlar and Andon, 2012). The adults, which are easier to scout

60 and identify, occur nocturnally during summer after root feeding has concluded and the grubs are
61 no longer present (Hallock, 1932). Current management options are limited and rely primarily on
62 the use of insecticides, yet products labeled for use against white grubs are either largely
63 ineffective (Krupke et al., 2007; DiFonzo 2007; Richer and Michel 2016) or have not yet been
64 evaluated specifically for this species in field cropping systems. It is commonly observed that
65 grubs are not adequately controlled by even high rates of neonicotinoid seed treatments (Krupke
66 et al., 2007; DiFonzo, 2007). Additionally, certain products like imidacloprid, thiamethoxam,
67 and carbaryl used against white grub species in turfgrass have been evaluated under laboratory
68 conditions and showed reduced efficacy against *M. formosae* relative to other white grub species
69 (Koppenhöfer and Fuzy, 2003; Koppenhöfer, 2010; Morales-Rodriguez et al., 2010;
70 Brandenburg and Freeman, 2012).

71 It is important to have effective sampling procedures to predict infestations prior to
72 planting, to be used in research on economic injury levels and action thresholds, and to
73 ultimately allow for the successful implementation of preventative pest management (Fleming
74 and Baker, 1936; Yates and Finney, 1942; Youngman et al., 1993; McLeod et al., 1999;
75 Youngman and Tiwari, 2004; Jordan et al., 2012). Annual white grubs are typically sampled
76 either using a pre-plant bait station to attract grubs, or by physically sifting grubs from a known
77 volume of soil (Fig. 3) (Youngman et al., 1993; Youngman and Tiwari, 2004; Jordan et al., 2012;
78 Tiwari et al., 2019). The bait station is a pre-plant method used to scout for grubs early season. It
79 works by planting corn on a wire mesh ledge a few weeks before the main crop and checking it
80 after the corn has germinated to identify the ground dwelling species that overwintered in the
81 field (Tiwari et al., 2019). This method provides a small window to identify and implement
82 management tactics in response to infestations.

83 Another way to sample for grubs is to use active soil sampling methods where the
84 contents of a known volume of soil are sifted and the contents analyzed. Three soil sampling
85 techniques have been evaluated to monitor white grub species including the golf hole cup cutter,
86 ‘standard’ and ‘compact’ soil cutter sampling methods. The golf hole cup cutter is primarily used
87 in the turfgrass industry and takes a cylindrical soil core with 10.16 cm diameter and 15.24 cm
88 depth (total volume = 1,236 cm³) (Koppenhöfer, 2010). The ‘standard’ and ‘compact’ soil cutter
89 methods take larger volumes of soil (9,439 and 4,195 cm³, respectively) from a broader surface
90 area but a shallower depth than the cup cutter (Jordan et al., 2012; Laub et al., 2018). Jordan et
91 al. (2012) determined that the latter two methods sample similar numbers of grubs per soil
92 volume and can predict spring grub population densities from fall sampling efforts in Virginia
93 corn-soybean rotated fields (Tiwari et al., 2019). The golf hole cup cutter has not yet been
94 evaluated for sampling white grubs in field cropping systems.

95 Prior to now there were no formal evaluations of sampling methods specifically for use
96 with *M. formosae* grubs in field cropping systems. The objective of this study is to identify
97 sampling methods that can both detect and sense varying densities of *M. formosae* grubs in corn-
98 soybean rotated fields in northwest Ohio. In this study we assessed soil sampling methods
99 including the ‘compact’ soil cutter, golf hole cup cutter, and a pre-plant baited station for *M.*
100 *formosae* grubs. Identification of sensitive sampling methods for *M. formosae* grubs will enable
101 farmers to prepare for future infestations and allow the best management strategies to be
102 evaluated and implemented.

103 **2. Materials and Methods**

104 Sampling methods for *M. formosae* grubs were evaluated from 2017-2019 in three agricultural
105 fields in northwest Ohio with known histories of *M. formosae* infestation (Table 1). At each

106 field, sampling occurred within 40 plots (30 x 30 m) arranged in an 8 x 5 grid that was
107 established over a 5-acre area where infestations were historically concentrated; the number of
108 plots used varied by experiment. Corn and soybean were grown following commercial
109 recommendations for the region with rows spaced 0.76 m and 0.38 m apart, respectively. Aside
110 from insecticide and fungicide seed treatments that are standard on commercial seed, additional
111 insecticide applications were not made. We compared two active sampling methods including
112 the golf hole cup cutter (Fig. 3A) (hereafter referred to as ‘cup cutter’), and compact soil cutter
113 (Fig. 3B) (hereafter referred to as compact cutter). Then, we evaluated the most successful
114 method against the bait station (Fig. 3C-D), a passive sampling method.

115 **2.1 Experiment 1 – Compact Cutter vs. Cup Cutter**

116 The cup cutter takes a cylindrical soil core with 10.16 cm diameter and 15.24 cm depth
117 (total volume = 1,235.56 cm³) (Koppenhöfer, 2010), while the compact cutter takes a larger
118 volume of 20.32 cm x 20.32 cm x 10.16 cm (total volume = 4,195.09 cm³) (Jordan et al., 2012).
119 In 2017, all 40 plots were sampled weekly for a total of four times between 18 May and 14 June
120 at the Fulton1 and Henry sites. By 21 June, we only observed pupae in our samples. On each
121 sampling date we took one cup cutter and one compact cutter sample, spaced 3 m apart, within
122 each plot such that each pair of samples was located approximately 30 m away from neighboring
123 pairs. We chose this design based on golf course scouting recommendations for white grubs used
124 to pinpoint areas of infestation (Grant, 1999; Dalthorp et al, 2000). We carefully sifted each soil
125 sample in a tray in the field and counted the number of *M. formosae* grubs per sample. We
126 recorded the number of *M. formosae* grubs (2nd and 3rd instars; Fig. 1B) in each sample.
127 *Maladera formosae* grubs are distinguished from other white grub species by their pronounced
128 maxillary stipes and aggressive behavior (Hallock, 1932)

129 **2.2 Experiment 2 – Bait Station vs. Cup Cutter**

130 The bait station design was adapted from Tiwari et al., (2019). Each station consisted of a
131 60.96 cm long by 7.62 cm wide wire-mesh (0.64 cm) strip bent medially at a 90-degree angle
132 into a 5.08 cm deep furrow. We placed 20 untreated corn seeds evenly along the bend in the trap,
133 then buried the trap with 2.54 cm soil and marked it with a flag. After two weeks, we evaluated
134 each bait station by pulling it from the soil, sifting the soil contents from within a 7.62 cm radius
135 around each station in a tray, and recording the number of grubs. At the time of bait station
136 removal, we took a paired cup cutter sample approximately 3 m from each station and counted
137 the number of grubs. We evaluated the cup cutter and wire-mesh bait station at the Fulton1,
138 Fulton2, and Henry sites in 2018, and at Fulton1 and Henry in 2019. At each site, we evaluated
139 both methods in 15 plots in 2018 and 10 plots in 2019. We set up all bait stations on 25 April in
140 2018 and 1 May in 2019. In 2018, the root and shoot lengths of each corn seedling from each
141 bait station, as well as the root-to-shoot length ratio, were recorded to assess feeding damage.
142 Few roots were damaged at each site during bait station removal across all sites; root losses were
143 negligible and did not bias the overall evaluation.

144 **2.3 Statistical Analysis**

145 **2.3.1 Experiment 1 - Compact Cutter vs. Cup Cutter**

146 Prior to analysis, we first adjusted the number of grubs sampled with the cup cutter by
147 multiplying by 3.395 to allow for equal comparisons based on soil volume since the two methods
148 sample different volumes of soil [the compact cutter takes a 3.395-times larger soil sample than
149 the cup cutter]. All statistical analyses were conducted using SAS 9.4 (SAS Institute Inc., 2013).
150 We assessed the grub count data per plot (from both the compact cutter and cup cutter) for

151 normality in separate analyses by site using PROC UNIVARIATE, and all datasets were non-
152 normal ($P \leq 0.05$). We performed a square root transformation ($[x + 0.5]^{1/2}$) on the adjusted grub
153 count datasets to account for their Poisson distributions and satisfy normality (Fry, 1999). In
154 separate analyses for Fulton1 and Henry, we compared the transformed number of grubs sampled
155 by the compact cutter to the cup cutter with respect to sampling date, sampling method, and
156 date*method interaction with a repeated measures analysis of variance using PROC MIXED. We
157 nested sampling method within plot, and designated block as a random effect. We performed
158 mean comparisons using Tukey-Kramer's post hoc test with LS means ≤ 0.05 .

159 We then determined whether grub captures from the two sampling methods were
160 correlated (i.e., do both methods reflect the same variations in grub population density). We
161 compared the average transformed capture rates for each compact cutter and adjusted cup cutter
162 sample taken per plot with robust linear regression analysis using PROC ROBUSTREG with an
163 M-estimation and default bisquare weighting function (i.e., non-zero residuals are somewhat
164 downweighted). We chose robust regression to identify and account for influential outliers in the
165 data set. Influential outliers are any data that significantly deviate from the other observations,
166 while leverage points are any datapoints in the X-Y plane that are distant from others but fall
167 within the extrapolated trendline (Chatterjee and Hadi 1986). When left unaccounted for, outliers
168 and leverage points can increase error rates and variance, reduce normality, and skew model
169 estimates (Osbourne and Overbay, 2004). We identified and accounted for zero influential
170 outliers and six leverage points in the Fulton1 dataset, and seven influential outliers and four
171 leverage points in the Henry dataset.

172 **2.3.2 Experiment 2 – Bait Station vs. Cup Cutter**

173 First, we assessed all grub count datasets for normality in separate analyses for each site-
174 year combination as described in Experiment 1. We determined that none of the datasets were
175 normally distributed, so we fit each dataset to various models using PROC GENMOD with a
176 log-link function and determined that the negative binomial model was optimal for both Fulton1
177 ($X^2 = 42.26$, $df = 47$, $P = 0.6688$) and Henry ($X^2 = 36.84$, $df = 47$, $P = 0.8607$) grub data sets. We
178 compared the average number of grubs sampled from the bait station and adjusted cup cutter of
179 each plot using PROC GLIMMIX with a negative binomial distribution and the independent
180 variables site, year, and the site*year interaction designated as fixed effects. We conducted mean
181 comparisons using a simulated LS means at a $P \leq 0.05$ level. Because no grubs were sampled at
182 Fulton2, we did not include it in the analysis. The number of grubs sampled by the bait station
183 and cup cutter was similar among site ($F = 1.80$, $df = 1, 96$, $P = 0.1830$) and year ($F = 2.16$, $df =$
184 $1, 96$, $P = 0.1449$), so we reran the final model with site and year designated as random effects
185 and sampling method as a fixed effect.

186 We conducted robust linear regression analysis with PROC ROBUSTREG to determine
187 whether the bait station and the cup cutter attracted/sampled grubs at similar rates. We used the
188 M-estimation and default bisquare weighting function. Prior to analysis, we performed a square
189 root transformation ($[x + 0.5]^{1/2}$) on the grub count dataset to satisfy normality. We aggregated
190 data from both sites since the site variable was previously found to be insignificant. Robust
191 regression analysis identified and accounted for two outliers and six leverage points in the
192 combined dataset.

193 We then evaluated bait station seedling plant growth characteristics datasets (i.e., root
194 length, shoot length, root-to-shoot length ratio) for normality by site with Box-Cox
195 transformation using PROC TRANSREG. We determined that the shoot length ($F = 7.43$, $df = 1$,

196 43, $P = 0.0092$) and root-to-shoot length ratio ($F = 48.00$, $df = 1, 43$, $P \leq 0.0001$) datasets had
197 estimated lambda values (i.e., an indication of the power to which data should be transformed to
198 satisfy the normality criterion) of zero and were log-transformed before further analysis. Root
199 length data ($F = 36.91$, $df = 1, 43$, $P \leq 0.0001$) had an estimated lambda of one and were not
200 transformed. We compared the average seedling root length, shoot length, and root-to-shoot
201 length ratio per bait station in separate analyses using PROC GLIMMIX with a gaussian
202 distribution model and site designated as a fixed effect. We conducted mean comparisons using a
203 simulated LS means at a $P \leq 0.05$ level. We included data from Fulton2 in the analyses to act as
204 a type of control (i.e., plants without grub feeding) to allow for a rough comparison. We are
205 hesitant to consider Fulton2 a true control since we did not account for potential site-specific
206 variation of local environmental conditions (e.g., precipitation, temperature, soil properties and
207 health, etc.).

208 **3. Results and Discussion**

209 *Maladera formosae* emerged as a significant pest of field corn in northern Ohio, northern
210 Indiana, and southern Michigan, within the last two decades (Krupke et al., 2007; DiFonzo,
211 2007; Hammond, 2013). Before this publication there were no formal evaluations of sampling
212 methods specifically for use with *M. formosae* grubs and adults in field cropping systems.

213 **3.1. Experiment 1 – Compact Cutter vs. Cup Cutter**

214 At Fulton1 (Fig. 4A), the number of grubs captured per plot varied significantly over time
215 ($F = 10.67$, $df = 3, 308$, $P < 0.0001$). We captured the most grubs per sample on 18 May, after
216 which populations significantly declined by 31 May and remained low for the last two sampling
217 dates. The cup cutter and compact method captured significantly different numbers of grubs per

218 soil volume overall ($F = 8.41$, $df = 1$, 308 , $P = 0.0040$). Overall, we sampled nearly twice as
219 many grubs per soil volume with the cup cutter method compared to the compact method. We
220 determined that differences in sampling method sensitivity were not dependent on time ($F =$
221 1.77 , $df = 3$, 308 , $P = 0.1527$), although multiple comparisons tests indicated that significantly
222 more grubs were captured per soil volume during the week of May 31.

223 At Henry (Fig. 4B), grub populations were approximately half of that observed at
224 Fulton1. The average number of grubs captured per plot was statistically similar across sampling
225 dates ($F = 1.06$, $df = 3$, 148 , $P = 0.3683$), although we sampled numerically more grubs on 18
226 May than at any other time during the study and observed the fewest grubs per sample on 14
227 June. Additionally, we sampled similar numbers of grubs per soil volume with the compact
228 cutter and cup cutter throughout the experiment ($F = 3.47$, $df = 1$, 148 , $P = 0.0646$). This trend
229 did not vary with time ($F = 1.25$, $df = 3$, 148 , $P = 0.2945$).

230 We observed a significant correlation between the cup cutter and compact cutter at both
231 sites (Fig. 5). The strength of the relationship varied with site. At Fulton1 (Fig. 5A) where
232 sampled grub populations were higher, there was a weaker, albeit significant, relationship
233 between the two methods ($R^2 = 0.1$, $X2_{(1,40)} = 5.70$, $P = 0.0170$). The cup cutter sampled 0.76
234 grubs per soil volume for every grub sampled by the compact cutter. However, when the
235 compact cutter failed to detect grubs, the cup cutter is expected to sample at least 0.5 grubs. At
236 Henry (Fig. 5B), we identified a strong positive association between the number of grubs
237 sampled per soil volume by the compact cutter and cup cutter ($R^2 = 0.5$, $X2_{(1,20)} = 56.59$, $P <$
238 0.0001); the cup cutter sampled 1.74 grubs per soil volume for every grub sampled by the
239 compact cutter. These findings indicate that both methods are reflecting the underlying grub

240 populations proportionally to each other, but that the cup cutter may be more sensitive to smaller
241 grub populations than the compact cutter.

242 The main difference between the two methods aside from sample area is sample depth;
243 the cup cutter samples 15.2 cm below the soil surface, or 5.1 cm deeper than the compact cutter.
244 This is relevant because *M. formosae* grubs are known to occur further below the soil surface
245 than other annual white grub species (Tashiro, 1987). Often, we observed grubs in the soil at the
246 bottom of the hole from a cup cutter, suggesting they are even further down than what we were
247 able to sample (personal observation, ALR, AJP). However, we do not know the relationship
248 between *M. formosae* grub depth and feeding damage to corn seedlings. In turfgrass, *M.*
249 *formosae* grubs are known to feed on roots lower underground than other grub species, causing
250 less damage, thus warranting a high action threshold of 18-20 grubs per 0.1 m² (Vittum et al.,
251 1999, Niemczyk and Shetlar, 2000, Morales-Rodriguez et al., 2010). Do grubs preferentially feed
252 closer to the surface on corn than they do in other systems like turfgrass? Feeding on nodal roots
253 close to the kernel would eliminate the primary root system and ruin plant development, whereas
254 feeding on lower lateral roots might allow for plant survival. Further work is needed to
255 determine how grub density and depth influences damage to the crop. Additionally, it is
256 unknown whether the cup cutter can be used to predict spring populations from fall sampling.
257 Researchers in Virginia determined that the compact method can predict white grub populations
258 in the spring from fall sampling (Jordan et al., 2012); in that system thresholds have been
259 established for annual white grubs (including *M. formosae*) which can aid in predicting future
260 economic loss.

261 **3.2. Experiment 2 – Bait Station vs. Cup Cutter**

262 In the second experiment, we confirmed that both the bait station and cup cutter could
263 detect early-season *M. formosae* grubs, and that the bait station attracted significantly more grubs
264 than the cup cutter was able to sample ($F = 4.68$, $df = 1$, 96 , $P = 0.0330$). In general, we observed
265 nearly 2.5 times more grubs around the bait station than in our cup cutter samples that were taken
266 alongside bait station (Fig. 6). We also detected a correlation in grubs recovered from paired
267 samples between the bait stations and cup cutters (Fig. 7) ($R^2 = 0.25$, $X^2_{(1,50)} = 38.31$, $P <$
268 0.0001). We observed 1.1 grubs per bait station for every grub sampled with the cup cutter,
269 suggesting that both methods are sensitive to the underlying grub population size.

270 Despite its ability to attract grubs and detect early-season infestations, the bait station has
271 several inherent disadvantages compared to the cup cutter. The bait station requires more
272 material and is difficult to set up and time consuming as it requires two trips the field for setup
273 and then breakdown and evaluation 1-2-weeks later, depending on growing conditions. It is
274 possible that the bait station attracted grubs from the surrounding soil that would otherwise have
275 been captured by the cup cutter. Regardless, the cup cutter requires just one trip to the field and
276 yields quick results in real-time relative to the bait station. Thus, it may be in the interest of the
277 crop professional (farmer, crop consultant, extension educator, researcher, etc.) to use an active
278 sampling approach that allows for systematic assessments and saves time and effort.

279 One advantage of the bait station is that it provides additional information with respect to
280 corn seedling growth characteristics/damage in the face of varying grub density. The average
281 corn seedling root length ($F = 38.22$, $df = 2$, 42 , $P < 0.0001$), shoot length ($F = 10.54$, $df = 2$, 42 ,
282 $P = 0.0002$), and root-to-shoot length ratio ($F = 28.43$, $df = 2$, 42 , $P < 0.0001$) of each bait station
283 significantly varied among sites (Fig. 8), with the most robust root and shoot growth at the
284 location with the fewest grub (Fulton2). We hesitate to consider Fulton2 a true control since each

285 field experienced different microclimatic variables (e.g., temperature, precipitation, secondary
286 pests, etc.) that were not accounted for during this study. Nonetheless, these findings suggest that
287 in the absence of grubs, seedlings at Fulton2 had longer roots and shoots than at Henry or
288 Fulton1 where the average number of grubs per bait station was 0.7 and 2.9, respectively. The
289 root-to-shoot length ratio was approximately 1 at Fulton2, but less than 1 at Henry and Fulton1
290 where roots comprised a smaller proportion of the total plant length. Regardless, future research
291 is needed to assess the value of bait stations for evaluating the relationship between plant growth
292 characteristics and grub density. It is also worth noting that the bait station and cup cutter
293 detected other soil-dwelling insect pest species in addition to *M. formosae* including other annual
294 white grub species like *Amphimallon majale* (Razoumowsky), *Anomala orientalis* Waterhouse,
295 *Phyllophaga* sp. Harris, *Popillia japonica* Newman, in addition to wireworms (Coleoptera:
296 Elateridae). It may be worthwhile to set up given the pest history of a given field.

297 **4. Conclusions**

298 This study reports the first evaluations of the compact cutter, cup cutter, and wire-mesh
299 bait station as sampling tools specifically for *M. formosae* grubs in field corn of the Great Lakes
300 region. We confirmed that both active soil sampling and passive bait station methods adequately
301 detect *M. formosae* grub populations. The cup cutter was more sensitive to grub density than the
302 compact cutter method, and requires fewer resources than the bait station. These results are
303 useful because they provide sampling standards for an emerging pest, which will contribute to
304 future studies on population biology, management tools, and economic thresholds – information
305 that is still greatly lacking for this pest (Hammond, 2013). Otherwise, these methods may be
306 beneficial for detecting and monitoring other agronomically relevant species.

307 **5. Author Contributions**

308 Conceptualization, AP, AR, ER, KT; Data curation, AP, AR, ER; Formal analysis, AP; Funding
309 acquisition, ER, KT; Investigation, AP, AR, ER, KT; Methodology, AP, AR, ER, KT; Project
310 administration, AP, KT; Resources, ER, KT; Software, KT; Supervision, AP, AR, ER, KT;
311 Validation, AP, AR, ER, KT; Visualization, AP; Roles/Writing – original draft, AP; Writing –
312 review & editing, AP, AR, ER, KT.

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318 **References Cited**

- 319 **Arrow, G.J. 1913.** Notes on the Lamellicorn Coleoptera of Japan and descriptions of a few new
320 species. Ann. Mag. Nat. Hist. Series 8, 12, 394–408.
321 <https://doi.org/10.1080/00222931308693416>
- 322 **Brandenburg, R.L., Freeman, C.P., 2012.** Handbook of Turfgrass Insects, 2nd edition.
323 American Phytopathological Society, St. Paul, MN.
- 324 **Chatterjee, S., Hadi, A.S., 1986.** Influential observations, high leverage points, and outliers in
325 linear regression. Stat. Sci. 1(3): 379-393.
- 326 **Dalthorp, D., Nyrop, J., Villani, M.G., 2000.** Spatial ecology of the Japanese beetle, *Popillia*
327 *japonica*. Entomol. Exp. Et Appl. 96: 129-139.
- 328 **DiFonzo, C., 2007.** Asiatic garden beetle in southern Michigan. MSU Extension field crops
329 news, 21 Jun 2007. Michigan State University, East Lansing, MI.
330 http://msue.anr.msu.edu/news/asiatic_garden_beetle_in_southern_michigan
- 331 **Fleming, W.E., Baker, F.E., 1936.** A method for estimating populations of larvae of the
332 Japanese beetle in the field. Jour. Agr. Res. 53: 319-331.
- 333 **Fry, J.C., 1999.** Biological data analysis: A practical approach. IRL Press, Oxford, UK.
- 334 **Grant, J.A., 1999.** Scarab grubs: Sampling and identification. Turfgrass Trends. 8(9): 1-6.
- 335 **Hallock, H.C., 1929.** Known distribution and abundance of *Anomala orientalis* Waterhouse,
336 *Aserica castanea* Arrow, and *Serica similis* Lewis in New York. J. Econ. Entomol. 22:
337 293-299.
- 338 **Hallock, H.C. 1930.** Some observations upon the biology and control of *Aserica castanea*
339 Arrow. J. Econ. Entomol. 23: 281-286.

- 340 **Hallock, H.C., 1932.** Life history and control of the Asiatic garden beetle. United States
341 Department of Agriculture Circular, Washington, D.C. 246: 1-16.
- 342 **Hallock, H.C. 1934.** The Asiatic garden beetle as a pest in vegetable gardens. J. Econ. Entomol.
343 27: 476-481.
- 344 **Hammond, R., 2013.** Asiatic garden beetle could be cause for concern for northern Ohio corn.
345 The Ohio State University, Wooster, OH. [https://cfaes.osu.edu/news/articles/asiatic-](https://cfaes.osu.edu/news/articles/asiatic-garden-beetle-could-be-cause-for-concern-for-northern-ohio-corn)
346 [garden-beetle-could-be-cause-for-concern-for-northern-ohio-corn](https://cfaes.osu.edu/news/articles/asiatic-garden-beetle-could-be-cause-for-concern-for-northern-ohio-corn)
- 347 **Jordan, T.A., Youngman, R.R., Laub, C.L. Tiwari, S., Kuhar, T.P., Balderson, T.K. Moore,**
348 **D.M., Saphir, M., 2012.** Fall soil sampling method for predicting spring infestation of
349 white grubs (Coleoptera: Scarabaeidae) in corn and the benefits of clothianidin seed
350 treatment in Virginia. Crop Prot. 39: 57-62.
- 351 **Koppenhöfer, A.M., 2010.** An integrated approach to insect management in turfgrass: white
352 grubs. New Jersey Agricultural Experiment Station commercial turfgrass and landscape
353 maintenance publications. Rutgers University, New Brunswick, NJ.
354 <https://njaes.rutgers.edu/fs1009/>
- 355 **Koppenhöfer, A.M., Fuzy, E.M. 2003.** Biological and chemical control of the Asiatic garden
356 beetle, *Maladera castanea* (Coleoptera: Scarabaeidae). J. Econ. Entomol. 96: 1076-1082.
- 357 **Krupke, C., Obermeyer, J., Bledsoe, L., 2007.** A new field crops pest for Indiana: Asiatic
358 garden beetle. Purdue Cooperative Extension Service, pest & crop newsletter, 8 Jun 2007.
359 Purdue University, West Lafayette, IN.
360 <https://extension.entm.purdue.edu/pestcrop/2007/issue11/>

- 361 **Laub, C., Youngman, R., Jordan, T., Kuhar, T., 2018.** Compact soil sampling strategy for
362 white grubs. Virginia Cooperative Extension, 19 Dec 2018. Virginia Tech, Blacksburg,
363 VA. <http://pubs.ext.vt.edu/2802/2802-7027/2802-7027.html>
- 364 **McLeod, M., Weiss, M., Rice, M.E., 1999.** White grubs, pp. 115-117, In Steffey, K.L., Rice,
365 M.E., et al. (Eds.), Handbook of corn insects. Entomol. Soc. Am. Lanham, MD.
- 366 **Morales-Rodriguez, A., Ospina, A., Peck., D.C., 2010.** Variation in the laboratory
367 susceptibility of turf infesting white grubs (Coleoptera: Scarabaeidae) to biological,
368 biorational and chemical control products. Pest Manag. Sci. 66: 90-99.
- 369 **Niemczyk, H.D., Shetlar, D.J., 2000.** Destructive Turf Insects, 2nd ed. H.D.N. Books, Wooster,
370 OH.
- 371 **Osborne, J.W., Overbay, A., 2004.** The power of outliers (and why researchers should
372 ALWAYS check for them). Pract. Assess. Res. Evaluation. 9(9): 10-8.
- 373 **Potter, D.A., 1998.** Destructive Turfgrass Insects: Biology, Diagnosis, and Control. Ann Arbor
374 Press, Chelsea, MI.
- 375 **Reding, M.E., Klein, M.G., 2007.** Life history of Oriental beetle and other scarabs, and
376 occurrence of *Tiphia vernalis* in Ohio nurseries. J. Entomol. Sci. 42: 329-340.
- 377 **SAS Institute Inc., 2013.** Statistical Analysis System (SAS) Software Manual: Base SAS 9.4
378 procedures guide: statistical procedures. SAS Institute, Inc., Cary, NC.
- 379 **Shetlar, D.J., Andon, J., 2012.** Identification of white grubs in turfgrass. The Ohio State
380 University Extension, Columbus, OH. <https://ohioline.osu.edu/factsheet/hyg-2510>
- 381 **Tashiro, H., 1987.** Scarabaeid pests: subfamily Melolonthinae, pp. 156-192 In Turfgrass Insects
382 of the United States and Canada. Cornell University Press, Ithaca, NY.

- 383 **Tiwari, S., Laub, C.A., Youngman, R.R., 2019.** Asiatic garden beetle in field corn. Virginia
384 Cooperative Extension newsletter 444-108. Virginia Tech, Blacksburg, VA.
385 [https://vtechworks.lib.vt.edu/bitstream/handle/10919/88901/444-108%20%28ENTO-](https://vtechworks.lib.vt.edu/bitstream/handle/10919/88901/444-108%20%28ENTO-295P%29.pdf?sequence=1&isAllowed=y)
386 [295P%29.pdf?sequence=1&isAllowed=y](https://vtechworks.lib.vt.edu/bitstream/handle/10919/88901/444-108%20%28ENTO-295P%29.pdf?sequence=1&isAllowed=y)
- 387 **Vittum, P.J., Villani, M., Tashiro, H., 1999.** Turfgrass insects of the United States and Canada.
388 Cornell University Press, Ithaca, NY.
- 389 **Yates, F., Finney, D.J., 1942.** Statistical problems in field sampling for wireworms. *Ann. App.*
390 *Biol.* 29: 156-167. <https://doi.org/10.1111/j.1744-7348.1942.tb07583.x>
- 391 **Youngman, R.R., Tiwari, S., 2004.** Recent advances and developments in corn integrated pest
392 management. In: Horowitz A. R., Ishaaya, I. (eds) *Insect Pest Management*. Springer,
393 Berlin, Heidelberg. https://doi.org/10.1007/978-3-662-07913-3_9
- 394 **Youngman, R.R., Midgarden, D.G., Herbert Jr., D.A., Nixon, K. H., Brann, D.E., 1993.**
395 Evaluation of a pre-plant method for detecting damage to germinating corn seeds by
396 multiple species of insects. *Environ. Entomol.* 22: 1251-1259.
- 397

398 **List of Figures and Tables**

399 **Figure 1.** Life stages of the Asiatic garden beetle, *Maladera formosae*. Egg and first instar on a
400 penny (A), and second (bottom) and third (top) instars next to a dime (B). Photo credits: (A)
401 Chris DiFonzo, Michigan State University, (B) Adrian Pekarcik, The Ohio State University.

402 **Figure 2.** Signs and symptoms of *M. formosae* grub feeding on roots of corn seedlings. Active
403 second and third instar feeding on roots (A) causes plants to wilt and discolor (B), and ultimately
404 die causing corn stand losses sometimes in excess of 40% (C). Photo credits: A-C) Amy
405 Raudenbush, The Ohio State University.

406 **Figure 3.** Sampling methods evaluated for *Maladera formosae* grubs. Golf hole cup cutter (A),
407 compact cutter (B), and the wire-mesh bait station set in a 5.2 cm deep furrow with 20 corn seeds
408 placed equidistant from each other during setup, (C) and prior to breakdown after two weeks (D).

409 **Figure 4.** Average number of *Maladera formosae* grubs per sampling method (i.e., compact
410 cutter and cup cutter) for each sampling date at Fulton1 site near Wauseon, OH (A) and at Henry
411 site near McClure, OH (B). Uppercase letters represent significant differences ($P < 0.05$) in the
412 total number of grubs sampled by date. Lowercase letters symbolize significant differences
413 between the two methods on that specific date. Samples were not taken during the week of May
414 25 due to time limitations.

415 **Figure 5.** Robust linear regression assessing the relationship between the average number of
416 *Maladera formosae* grubs sampled per soil volume for each cup cutter and compact cutter pair
417 across all sampling periods in 2017 at Fulton1 (A) and Henry (B) sites in northwest Ohio.
418 Fulton1 and Henry were previously planted to soybean and corn, respectively. The relative size
419 of each datapoint corresponds to the number of samples with those values.

420 **Figure 6.** Average number of *Maladera formosae* grubs attracted to each bait station and
421 sampled by each cup cutter per plot from Fulton1 and Fulton2 sites in northwest Ohio in 2018
422 and 2019. Uppercase letters represent significant differences ($P < 0.05$).

423 **Figure 7.** The relationship between the number of *Maladera formosae* grubs sampled by or near each
424 cup cutter and bait station pair, respectively, evaluated at Fulton1 (X) and Henry (□) sites in northwest
425 Ohio in spring 2018 and 2019.

426 **Figure 8.** Average root (upper green bars) and shoot (lower tan striped bars) lengths per corn
427 seedling from each bait station after two weeks in the field at Fulton1, Fulton2, and Henry sites
428 in northwest Ohio in spring 2018. Uppercase and lowercase letters indicate significant
429 differences ($P < 0.05$) in shoot length and root length, respectively, among sites. The number of
430 grubs sampled per bait station at Fulton1, Fulton2, and Henry sites averaged one, zero, and three,
431 respectively.



432

433 **Figure 1. Color**

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435 **Figure 2. Color**

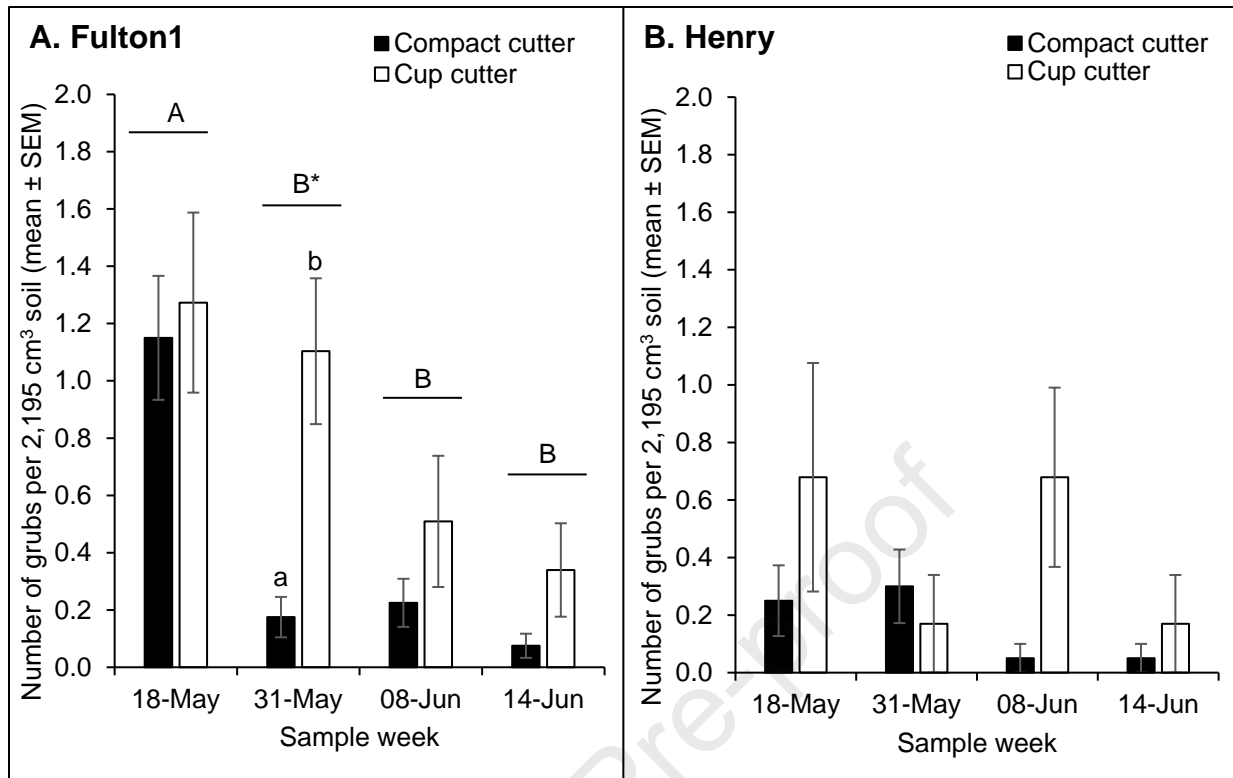
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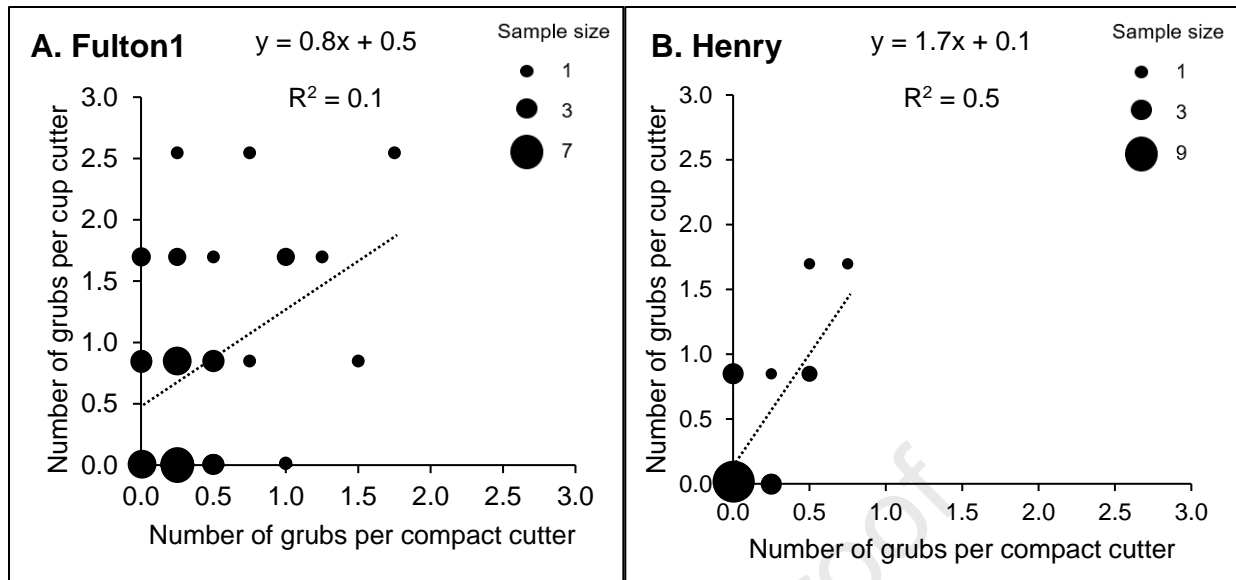
437 **Figure 3. Color**

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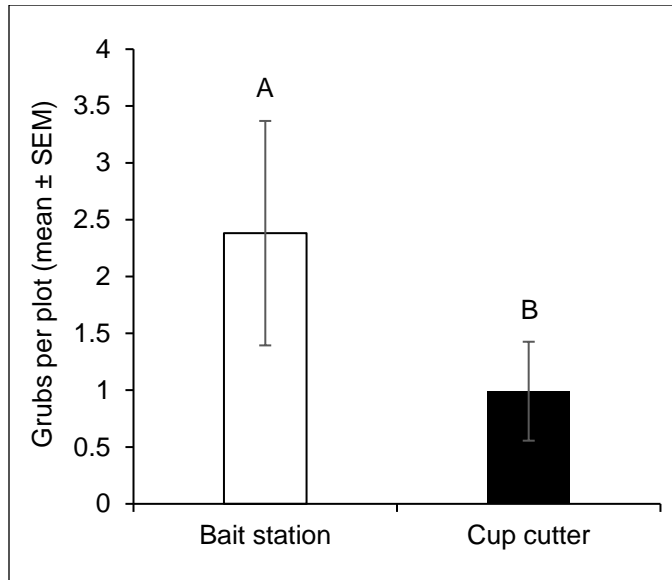
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439 **Figure 4.**



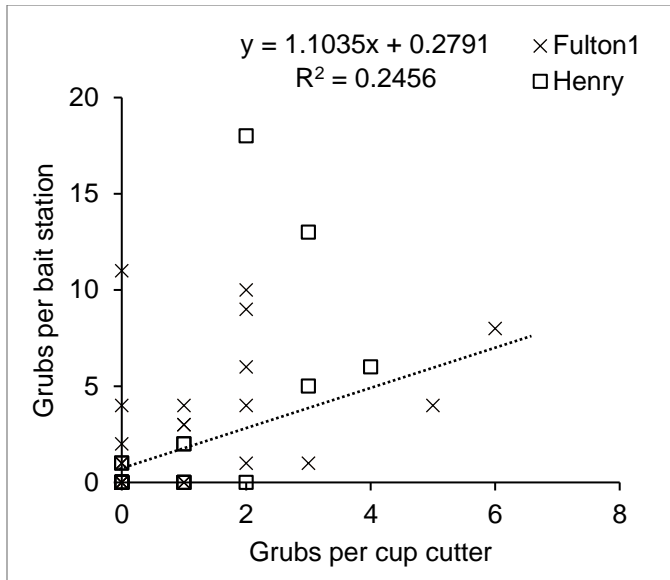
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441 **Figure 5.**



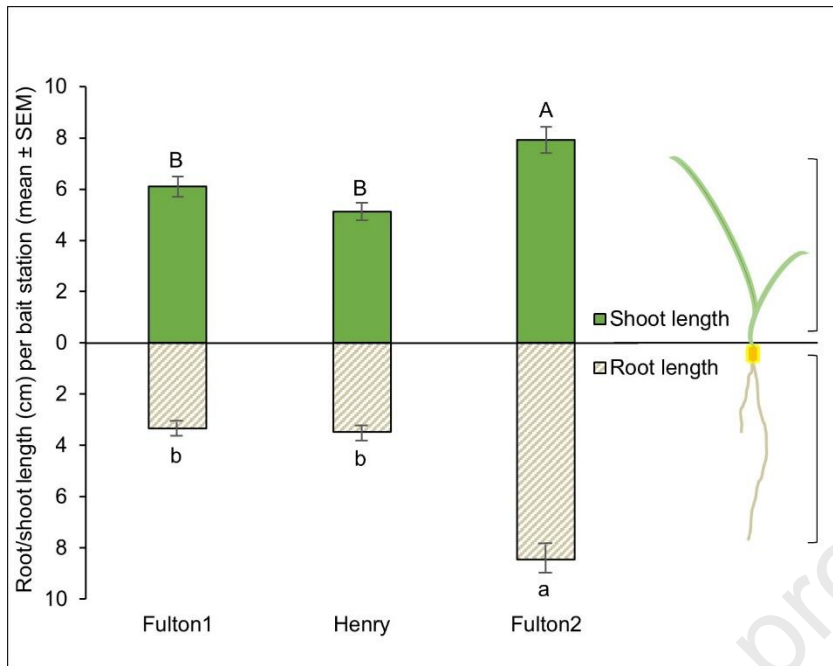
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443 **Figure 6.**



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445 **Figure 7.**



446

447 **Figure 8. Color**

448 **Table 1.** Field locations used for *Maladera formosae* grub sampling in northwest Ohio from
 449 2017 to 2019. Sampling method abbreviations are as follows: BS = wire-mesh bait station; CC =
 450 golf hole cup cutter; CS = compact soil cutter.

451

Site name	Latitude, Longitude	Nearest city/town	Year	Previous crop	Crop	Grub sampling methods
Fulton1	41.617739, -84.134506	Wauseon, OH	2017	Soybean	Corn	CM, CC
			2018	Corn	Soybean	CC, BS
			2019	Soybean	Fallow	CC, BS
Fulton2	41.599141, -84.145894	Wauseon, OH	2018	Corn	Fallow	CC, BS
Henry	41.385992, -83.960931	McClure, OH	2017	Soybean	Corn	CM, CC
			2018	Corn	Soybean	CC, BS
			2019	Soybean	Corn	CC, BS

Highlights

- Cup cutter is more sensitive to varying grub densities than compact cutter
- Bait station and cup cutter allow for passive pre-plant sampling of grubs in corn
- Cup cutter requires only one trip to the field and less supplies than bait station

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Declaration of interests

- The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
- The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

All authors have substantially contributed to drafting this manuscript and approve of it for submission as a research article. The authors have no competing interest We confirm that this manuscript has not been previously published and is not currently under consideration by any other journal. Additionally, each author has approved the entire content of this paper and have agreed to Crop Protection's submission policies. No funding was received for conducting this research. To the best of our knowledge, the named authors have no conflict of interest or permissions information, financial or otherwise.

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