

# Developing Sampling Plans to Estimate Asiatic Garden Beetle Damage in Commercial Mint

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## ABSTRACT

The Asiatic garden beetle (AGB) *Maladera castanea* Arrow has become a serious pest of commercial mint fields in Indiana. The larval (grub) stage feeds on mint roots, causing stunting and even plant death when feeding damage is severe, but the relationship between grub density and yield loss is unclear. We evaluated several sampling approaches over the course of the spring and fall of 2021 to optimize sampling intensity, characterize the grub species complex associated with mint, and resolve relationships between grub density and plant performance. Based on field level estimates of mean : variance ratios provided by the different sampling approaches, the best estimates of grub density, and below- and above-ground plant biomass were provided by randomly selecting and sampling 30, 0.25m<sup>2</sup> quadrats within a 0.2 ha grid composed of 64 total cells (0.003 ha/cell). White grub populations in the 3 mint fields were composed of 91% AGB, 8% Japanese beetle *Popillia japonica*, and 1% Masked chafer *Cyclocephala* spp. Grub densities varied seasonally and with field, and plant performance decreased significantly with increasing grub densities. Results to date provide a reliable sampling protocol for examining relationships between grub density and plant performance in mint and indicate that grub densities of 13/0.25 m<sup>2</sup> and higher may significantly reduce the performance of mint plants.

## INTRODUCTION

Indiana is one of the top mint-producing states in the U.S. ranking 3<sup>rd</sup> in spearmint production and 4<sup>th</sup> in peppermint production [3]. In recent years, invasive AGB grubs, arriving in New Jersey in 1921 from Japan and China [2], have been cited by Indiana mint growers as the top insect threat to their late-summer harvest. One mint grower has attributed a \$325 per acre loss to these insects [4]. Because the AGB's main damaging stage remains underground [1], it can be difficult for growers to pinpoint the problem in their crop. The aim of this study was to evaluate multiple sampling methodologies and then use the most effective approach to examine the relationship between AGB grub density and performance of mint plants in infested fields.

## OBJECTIVES

- To develop a reliable sampling methodology for quantifying white grub populations and plant performance in commercial mint fields.
- To characterize the relationship between AGB density and plant performance in commercial mint fields during different times of the growing season.

## METHODS

- Two sampling methods were compared in three fields during the spring. The first method used a single soil core (≈0.01m<sup>2</sup>) to estimate grub density within each plot, followed by measuring the height of the nearest plant, and assigning that plant a root rating based on an ordinal scale of 1-5. The second method involved collection of above-ground plant biomass within a single 0.25m<sup>2</sup> sampling quadrat in each plot, followed by excavation of the soil and plant roots to a depth of 10 cm, followed by collection and identification of all white grubs to provide estimates of above- and below-ground biomass, and grub density (Figure 1, D, E, & F).
- Sampling intensity was optimized using the mean : variance ratios provided by the spring sampling data to determine the lowest number of samples required to achieve stable estimates of grub density and plant performance. Mean : variance ratios were calculated for sample sizes ranging from 1 to 60 (1, 10, 20, 30, 40, 50, 60) by drawing samples randomly from the spring data set and repeating this process 5 times (n=5).
- Once optimal sampling intensity was established (n=30), the utility of systematic vs random sampling schemes (3 of each) was assessed using the difference between mean grub density and plant biomass estimated using each type of sampling scheme, and the true means estimated using all samples collected from each field.
- Fall sampling was conducted using 30 samples collected at random from the 64-cell grid as validated by the above comparisons.

## RESULTS

- The sampling method employing the 0.25 m<sup>2</sup> quadrat provided more stable estimates of grub density compared to the 0.01 m<sup>2</sup> soil corer.
- Sampling intensity was reduced to 30 samples per field based on the leveling-out of sample variance at that point for each response variable (Figure 2).
- A random sampling scheme provided more accurate and less variable estimates of response means compared to systematic sampling schemes (Figure 3).
- Grub densities varied seasonally with 2 of the 3 fields experiencing higher mean densities during the fall (Table 1, Figure 5).
- Overall, root : shoot biomass ratio increased as AGB larval density increased, but this was primarily driven by decreases in above ground plant biomass. Significant decreases in below-ground plant biomass were not detected, even in heavily infested plots (Table 1, Figure 4).
- Both plant height and root condition declined with increasing grub density in the field experiencing the highest grub densities during the spring (Table 1).

## DISCUSSION

- Refining the sampling methodology allowed us to accurately estimate grub densities and quantify plant damage while reducing overall sampling effort.
- Despite the shortcomings of the soil coring approach (0.01 m<sup>2</sup>), this approach did reveal that plant height and root rating were both negatively affected by increasing AGB larval densities.
- Surprisingly, AGB's negative impact on plant biomass was evident only above-ground, perhaps as a result of damage to fine root hairs that may mediate moisture and nutrient acquisition even when damage to these fine root structure could not be detected by measuring below-ground biomass under field conditions.
- Preliminary results indicate that Asiatic garden beetle densities of 13/0.25 m<sup>2</sup> may significantly reduce mint yields.

# Random sampling reveals potential economic injury level for Asiatic garden beetle in mint



**Figure 1, A:** The Asiatic garden beetle life cycle starting from the far-right going clockwise: larva (grub), pupa, and adult, **B:** Plant damage resulting from AGB larvae infesting a spearmint field, **C:** Evidence of root feeding from larvae of the Asiatic garden beetle compared to a healthy root system (right), **D:** Sampling method using a soil coring device to (0.01 m<sup>2</sup>) estimate grub density, **E:** Sampling method using a 0.25m<sup>2</sup> quadrat to estimate above-ground biomass, which was harvested just before excavating the soil, **F:** Sampling method using a 0.25m<sup>2</sup> quadrat to excavate soil and estimate below-ground biomass and grub density.

## References:

- Capinera, J. L. 2020. Order Coleoptera—Beetles, White Grubs, and Wireworms, pp. 63-204. Handbook of Vegetable Pests, 2<sup>nd</sup> ed. Academic Press.
- Hallock, H. C. 1936. Life History and Control of the Asiatic Garden Beetle. In H. C. Hallock and I. M. Hawley (eds.). USDA Circular, Washington, D.C.
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## Acknowledgements:

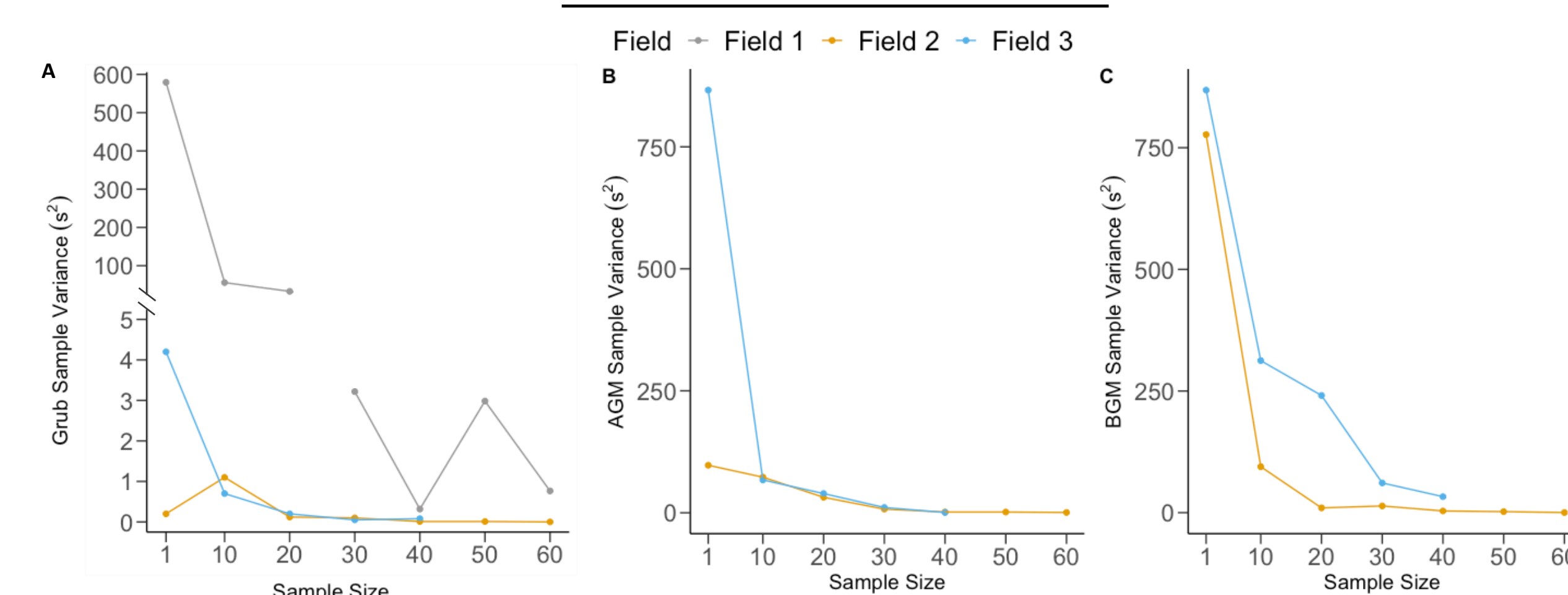
- Purdue Soil Insect Ecology Lab
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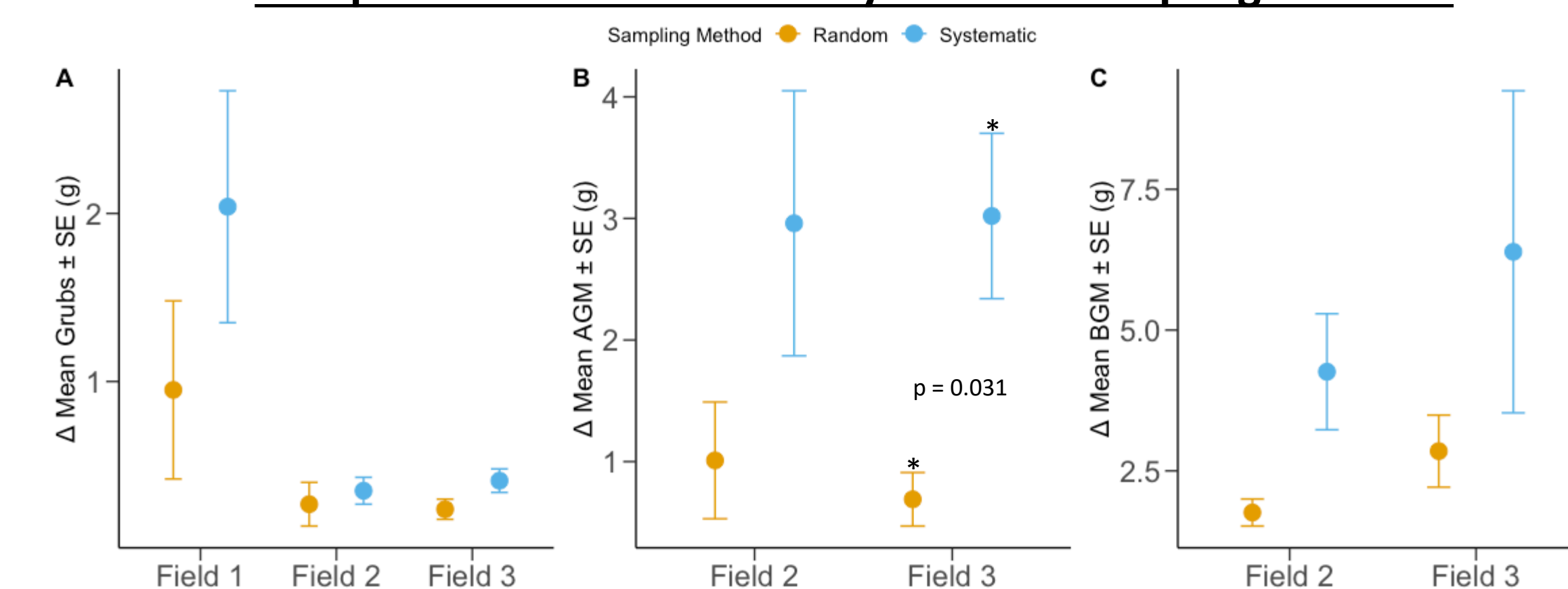
## FIGURES

### Mean : variance ratio results



**Figure 2:** Sample variance among 5 replicates for the collection of (A) grubs, (B) above-ground biomass (AGM), and (C) below-ground biomass (BGM) (C) at increasing sample sizes.

### Comparison of random and systematic sampling schemes

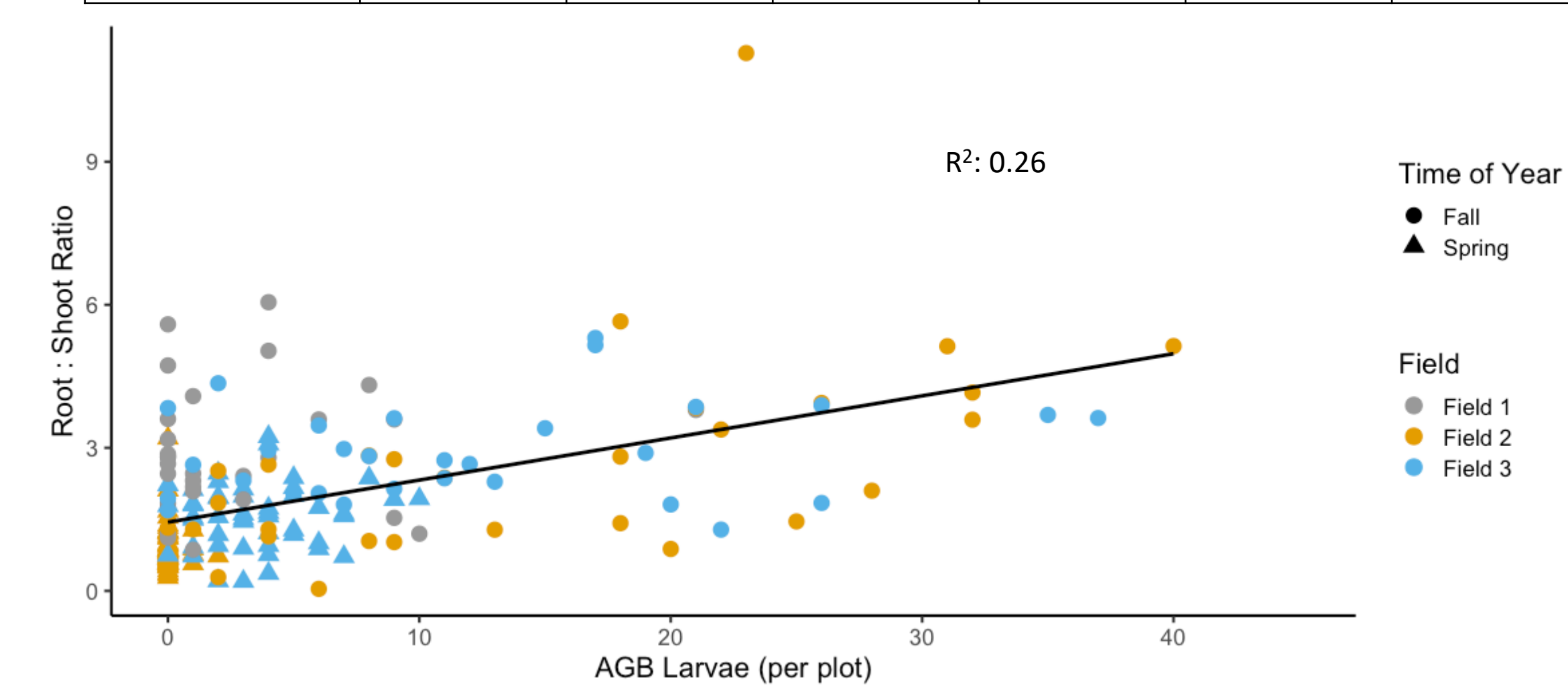


**Figure 3:** The difference between the estimated provided by random and systematic sampling schemes and the true mean estimated using all samples collected from a given field (A) grub density, (B) above-ground biomass (AGM), and (C) below-ground biomass (BGM). \* indicates a significant difference between sampling methods within a given field.

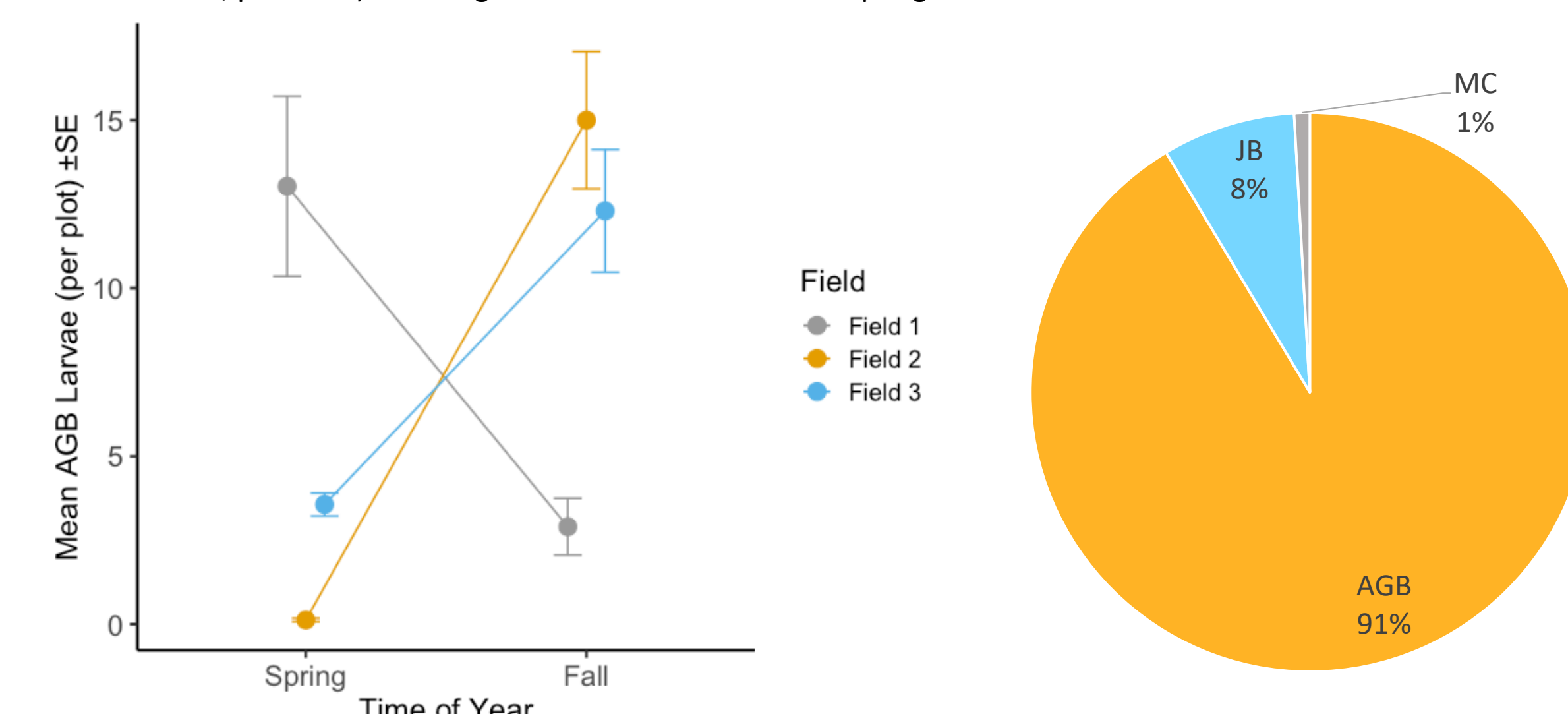
### Results of AGB effect on plant performance in 2021

**Table 1:** The mean Asiatic garden beetle (AGB) grub density per plot in each field in the spring and fall as well as the regression results for the relationship between AGB grub density and above- and below-ground biomass (g), root : shoot ratio, plant height (cm), and root rating (1-5). Reported are the t-values, p-values, and R<sup>2</sup> values for each regression. Letters adjacent to AGB density means indicate significant differences between fields. N/A indicates the data was not recorded in the corresponding field.

	Spring			Fall		
	Field 1	Field 2	Field 3	Field 1	Field 2	Field 3
Mean AGB/0.25m <sup>2</sup> (±SE)	13.03 ± 2.68 b	0.13 ± 0.05 a	3.56 ± 0.34 a	2.9 ± 0.89 a	15.00 ± 2.04 b	12.3 ± 1.83 b
Above-ground biomass (g)						
t	N/A	0.10	0.89	-0.81	-4.08	-0.98
p	N/A	0.921	0.379	0.424	<0.001	0.337
R <sup>2</sup>	N/A	<0.01	0.02	0.02	0.37	0.03
Below-ground biomass (g)						
t	N/A	0.26	0.86	0.19	0.26	0.15
p	N/A	0.793	0.396	0.849	0.799	0.880
R <sup>2</sup>	N/A	<0.01	0.02	<0.01	<0.01	<0.01
Root : Shoot ratio						
t	N/A	-0.24	0.56	0.74	3.29	1.20
p	N/A	0.809	0.577	0.469	0.003	0.239
R <sup>2</sup>	N/A	<0.01	0.01	0.02	0.29	0.05
Plant height (cm)						
t	-2.18	N/A	N/A	N/A	N/A	N/A
p	0.033	N/A	N/A	N/A	N/A	N/A
R <sup>2</sup>	0.06	N/A	N/A	N/A	N/A	N/A
Root Rating (1-5)						
t	6.79	N/A	N/A	N/A	N/A	N/A
p	<0.001	N/A	N/A	N/A	N/A	N/A
R <sup>2</sup>	0.43	N/A	N/A	N/A	N/A	N/A



**Figure 4:** Relationship between Asiatic garden beetle (AGB) larval density and root : shoot ratio (F<sub>1,198</sub> = 71.24, p < 0.001) including data from all 3 fields in the spring and in the fall.



**Figure 5:** Mean Asiatic garden beetle (AGB) larvae per plot during the spring and fall sampling periods. Seasonal differences in grub density vary with field (season x field interaction F<sub>2,260</sub> = 24.68, p < 0.001). See Table 1 for pairwise comparisons between each field

**Figure 6:** Total white grub species compositions shown as a percentage for the year 2021 consisting of Asiatic garden beetle (AGB), Japanese Beetle (JB), and masked chafer (MC).