

Managing pests by increasing predators, and improving agroecosystem quality through late termination of cover crops

Introduction

With growing popularity of delaying cover-crop termination and planting green, there is a need to understand effects of delayed cover-crop termination on pest control in no-till agronomic systems. In this report, I quantify in no-till corn systems in 16 locations (Delaware, Florida, Iowa, Illinois, Kansas, Kentucky, Maryland, North Carolina, Nebraska, New York, Ohio, Pennsylvania, Texas, Virginia, Virginia Tech, and Wisconsin) effects of differential timing of termination of a cereal-rye (*Secale cereal* L.) cover crop on pest and predator communities. With this framework, I ask the question: How do sentinel prey rates change among treatments and growth stages, and is there an interaction between the two? I hypothesized that by increasing overall cover-crop biomass (ecosystem complexity) and using IPM (i.e., avoiding preventative insecticides), I would record higher levels of predation.

Study design

In a three-year field experiment (2021-2023) across sixteen collaborating locations (typically a University) at their respective research stations, we established a single-factor experiment, arranged in a randomized complete block design with four cover crop treatment levels and five-six replicates. Plots were 12 x 15 m with 16 crop rows at 30 inch spacing. Cover-crop treatments varied by the timing of termination of a cereal-rye cover crop and included 1) a no cover-crop control (Referred to here as ‘No CC’) and cover-crop terminations of: 2) 14-28 days pre-planting (DPP; referred to here as ‘early terminated’), 3) 3-7 DPP (‘late terminated’), and 4) 1-3 days after planting (DAP; ‘planting green’) of the cash crop. Cover crops were terminated using glyphosate (1.27 kg ae ha⁻¹) and ammonium sulfate (2.5% v/v) using water as a carrier at 140 L ha⁻¹. Based on abiotic conditions, planting dates of cover and cash crops, and termination dates varied annually among locations. We established a 3-m border of cover crop around the experiment and 9 m alleys between blocks. The border and the alleys follow the planting-green schedule for termination and cash-crop planting (Fig. 1).

To assess the influence of the timing of cover-crop termination on predation by generalist invertebrate predators, I conducted sentinel prey assays with waxworms (*Galleria mellonella*; Timberline Fisheries, Marion, IL) in corn across growth stages (Lundgren et al. 2006; Busch et al. 2020). We pinned waxworms through their terminal segment into balls of modelling clay, which we laid on the soil surface. We deployed six waxworms per plot in vertebrate exclusion cages made from hardware cloth (4-mm mesh) and capped with a plastic lid that allows for free passage of predatory invertebrate species. We deployed three waxworms per row at ~8:00 in two rows of each plot (between rows 6 and 7 and 11 and 12, approximately 4, 8, and 12 m from the western edge of each plot; total of 6 cages per plot). We checked on caterpillars at ~20:00 and again the next day at ~8:00, recording presence/absence and noting any predators (e.g., wolf spider, carabid beetle, ant, etc.) that were present. Missing waxworms were not replaced with living ones.

Statistical Procedures

I conducted all statistical analyses in R (version 4.4.1). On several occasions observations were missing due to trap disturbances in the field. These were recorded as 'NA' and removed during the wrangling process. Summary statistics for predation at the treatment and growth stage levels are in Table 1. This table was created using the Mosaic package (Pruim et al., 2017). The estimated mean probabilities for growth stages V3 and V5 were similar, at 0.72 and 0.79, respectively. The estimated mean probability for growth R3 was higher than both V3 and V5 at 0.9. The number of observations per growth stage varied slightly, $n_{V3} = 3902$, $n_{V5} = 4196$, and $n_{R3} = 4334$, respectively. While this is unbalanced, there are many observations within each growth stage. At the treatment level, the estimated mean probabilities for No CC, early-termination, late termination, and planting green are relatively similar, 0.77, 0.8, 0.81, and 0.86, respectively. Sample sizes varied, $n_{Tt1} = 3439$, $n_{Tt2} = 3203$, $n_{Tt3} = 2605$, and $n_{Tt4} = 3185$, respectively. Again, while unbalanced, there are many observations within each treatment.

The response variable was the binomial of predation with 1 referring to predation (success) and 0 referring to no predation (failure). The fixed effects terms for this model were treatment, growth stage,

and their interaction. To account for the repeated measure structure of sampling six traps from the same plot, I developed a nested random effects term with plot within block within location within year (Fig. 2). Figure 2 was created using the DiagrammeRsvg package (Iannone, 2016). The final data frame size used in the model(s) had 12432 observations of plot:(block:(location:year)), 744 observations of block:(location:year), 39 observations of location:year, and 3 observations of year.

A density plot to visualize the data before modeling was also created using the Mosaic package (Fig. 3, Pruim et al., 2017). This was done to ensure no instances of 100% success (predation) or failure (no predation). To analyze effects of timing of cover-crop termination, I ran a generalized linear mixed-effect model (GLMM) on sentinel prey (binomial) data. Model refinement was done using a Type-II Wald- X^2 test with P-values, derived from the car package (Weisberg, 2019). All models were followed by effects plots (effects package) on both the response and link scale and pairwise Tukey-comparisons using the emmeans package (Fox and Hong, 2009; Lenth, 2024). R-squared values were derived from the performance package (Lüdtke et al., 2021).

To answer the question of interest, it was necessary to determine if the interaction between treatment and growth stage was needed in the model. After an initial model, I conducted a Type-II Wald- X^2 test, and using the P-values, interpreted the effect of the interaction term ($X^2(6) = 8.25$, $P\text{-value} = 0.22$). With this initial model, I can conclude that there is weak evidence against the null hypothesis of no interaction between treatment and growth stage on total predation, after controlling for plot nested in block nested in location nested in year, and will remove it from the model.

Results

The final theoretical model was: $\pi(\text{Predation}|\text{Treatment}, \text{GrowthStage}) \sim \beta_0 + \beta_1 I_{\text{Treatment}=2i} + \beta_2 I_{\text{Treatment}=4i} + \beta_3 I_{\text{Treatment}=3i} + \beta_4 I_{\text{GrowthStage}=V5i} + \beta_5 I_{\text{GrowthStage}=V3i} + \text{Year}_i + \text{Location}_i + \text{Block}_i + \text{Plot}_i + \text{Error}_i$

where treatment was a four-level categorical variable represented by the indicator variables $I_{Treatment=2}$ (which takes on a value of 1 if the treatment is 2, and 0 if not), $I_{Treatment=3}$ (which takes on a value of 1 if the treatment is 3, and 0 if not), $I_{Treatment=4}$ (which takes on a value of 1 if the treatment is 4, and 0 if not), and growth stage was a three-level categorical variable represented by indicator variables $I_{GrowthStage=V3}$ (which takes on a value of 1 if the growth stage is V3, and 0 if not), and $I_{GrowthStage=V5}$ (which takes on a value of 1 if the growth stage is V5, and 0 if not). This means that the first treatment level (No CC) and the third growth stage (R3) are treated as references.

A Type-II Wald- X^2 test was generated to assess the model. Accounting for growth stage, year, location, block, and plot, there is very strong evidence against the null hypothesis of no differences among treatment ($X^2(3) = 60.988$, $P\text{-value} < 0.0001$), so we would conclude that there is some difference in the estimated log differences of total predation across treatments. Accounting for treatment, year, location, block, and plot, there is very strong evidence against the null hypothesis of no differences among growth stages ($X^2(2) = 528.43$, $P\text{-value} < 0.0001$), so we would conclude that there is some difference in the estimated log differences of total predation across growth stages. The model has a conditional R-squared of 0.528 and a marginal R-squared of 0.083. This means that the random effects explain 52.8% of the variation on total predation and treatment and growth stage explain 8.3% of the variation on total predation. After we account for treatment and growth stage, the estimated correlation (ICC) at the plot level was 0.236. This indicates a low correlation between two observations once we account for systematic changes across plots. To visualize the effects of treatment and growth stage on the log-link and response scale, effects plots are displayed in Figure 4. In Figure 4, panel *a* is on the log-link scale and panel *b* is on the response scale. Figures 5 and 6 display the raw data estimated probabilities with compact-letter display derived from the post-hoc emmeans Tukey-comparisons on the response scale.

For two otherwise similar plots that differ by treatment, there is an estimated probability of 0.88 of predation in the No CC-treatment (95% CI: 0.787 to 0.935), an estimated probability of 0.895 of

predation for the early-terminated treatment (95% CI: 0.812 to 0.944), an estimated probability of 0.918 of predation for the late-terminated treatment (95% CI: 0.849 to 0.957), and an estimated probability of 0.938 of predation for the planting-green treatment (95% CI: 0.884 to 0.968), controlling for growth stage, year, location, block, and plot. For two otherwise similar plots that differ by growth stage, there is an estimated probability of 0.828 of predation for growth stage V3 (95% CI: 0.717 to 0.902), an estimated probability of 0.893 of predation on growth stage V5 (95% CI: 0.814 to 0.941), and an estimated probability of 0.963 of predation for growth stage R3 (95% CI: 0.931 to 0.980), controlling for growth stage, year, location, block, and plot..

Scope of Inference

The locations chosen for this experiment were not randomly selected (Universities volunteered to join the project), and thus the results would not generalize beyond the locations present. Additionally, the results only apply to the years 2021-2023 in a corn monoculture following a cereal-rye cover crop. Data were collected at three, non-random time points throughout the season, and we cannot generalize beyond those time points. And so, we cannot infer results on other locations that do not fall within this range of years, crop, nor sampling time points. In-field location of plots, however, was randomly assigned and thus we can infer on these respected locations the effects of treatment on predation. Furthermore, we can make causal inference on predation levels at the treatment level. Keeping these limitations in mind, the study showed that by delaying cover-crop termination, there was an increase in predation. Additionally, as the season persisted (V3-V5-R3), there was an increase in predation. Based on Figure 5 we see treatment impacts total predation that with delayed cover-crop termination there is an increase in the estimated proportion of predation. Based on Figure 6 we see growth stage impacts total predation that as the season persists (V3-V5-R3), there is an increase in the estimated proportion of predation.

Figures

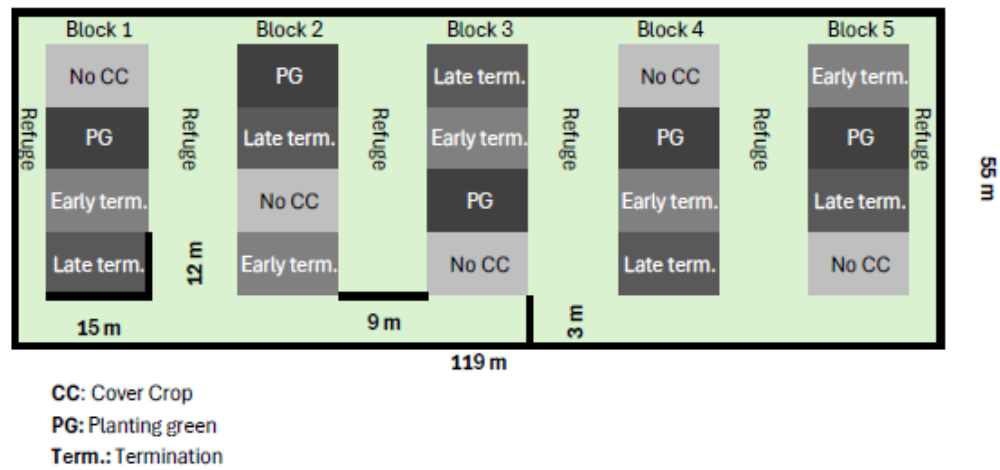


Figure 1. Example schematic diagram of the design showing treatments, blocks, and buffer areas. The treatment layouts may differ by location and year of the experiment. (Early: 14-28 DPP; Late: 3-7 DPP; Green: 1-3 DAP)

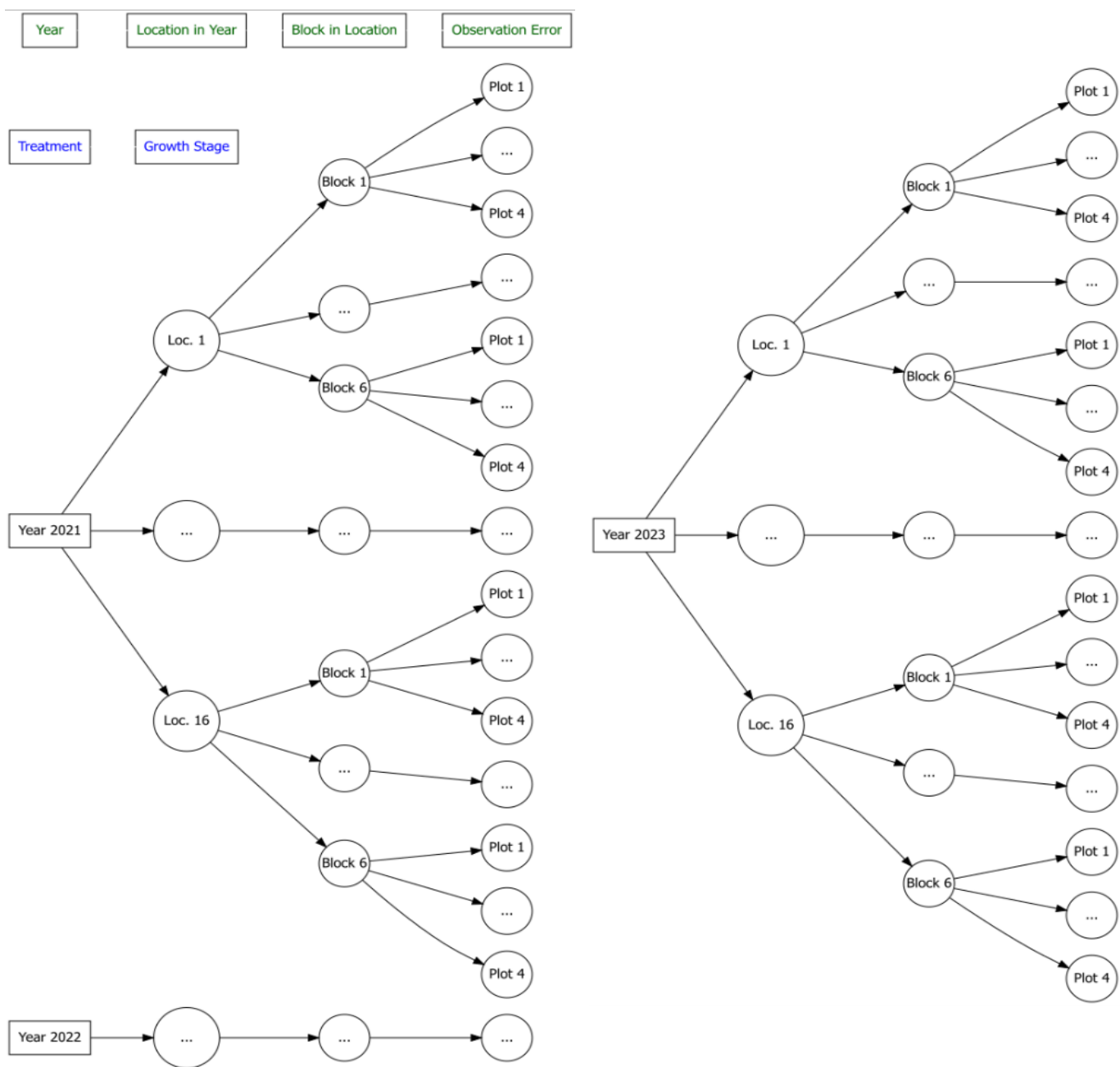


Figure 2: Mapped out random effects hierarchy. This figure displays the nested structure for the plot within block within location within year design.

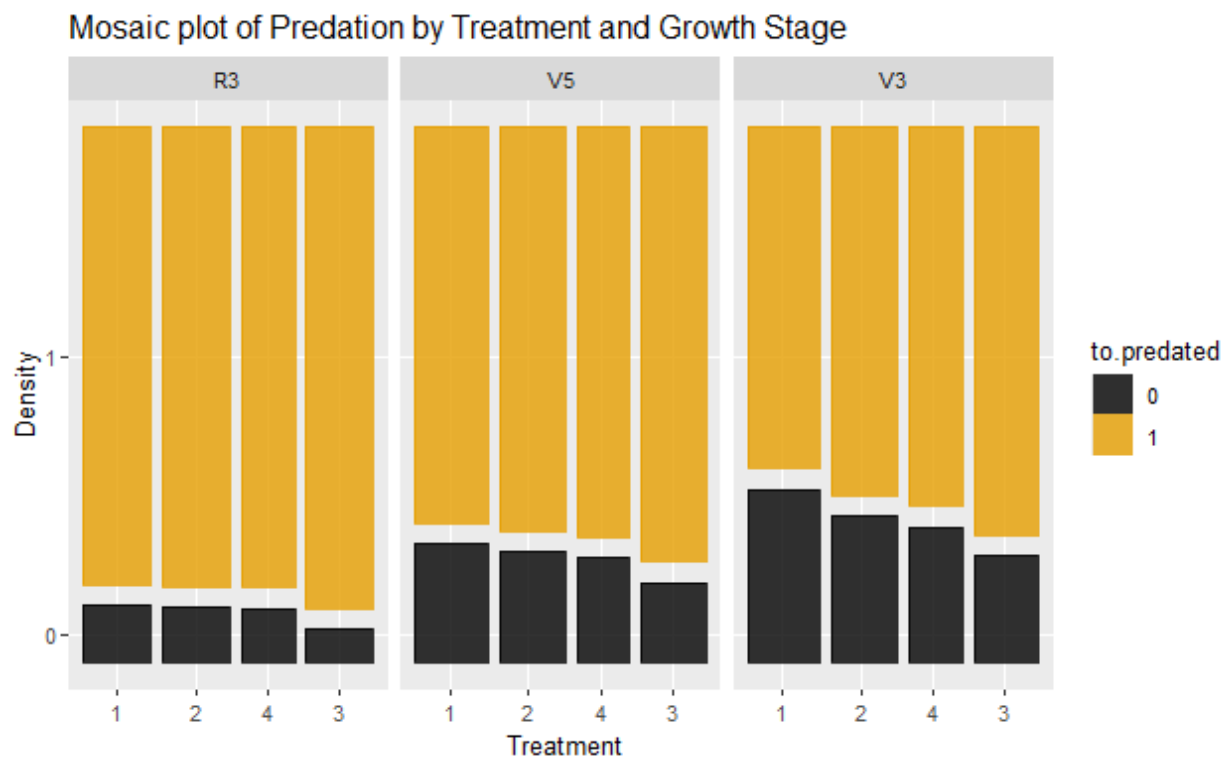


Figure 3: Density plot of the raw response variable across the treatment and growth stage predictors. Yellow represents a success (predation) and black represents a failure (no predation).

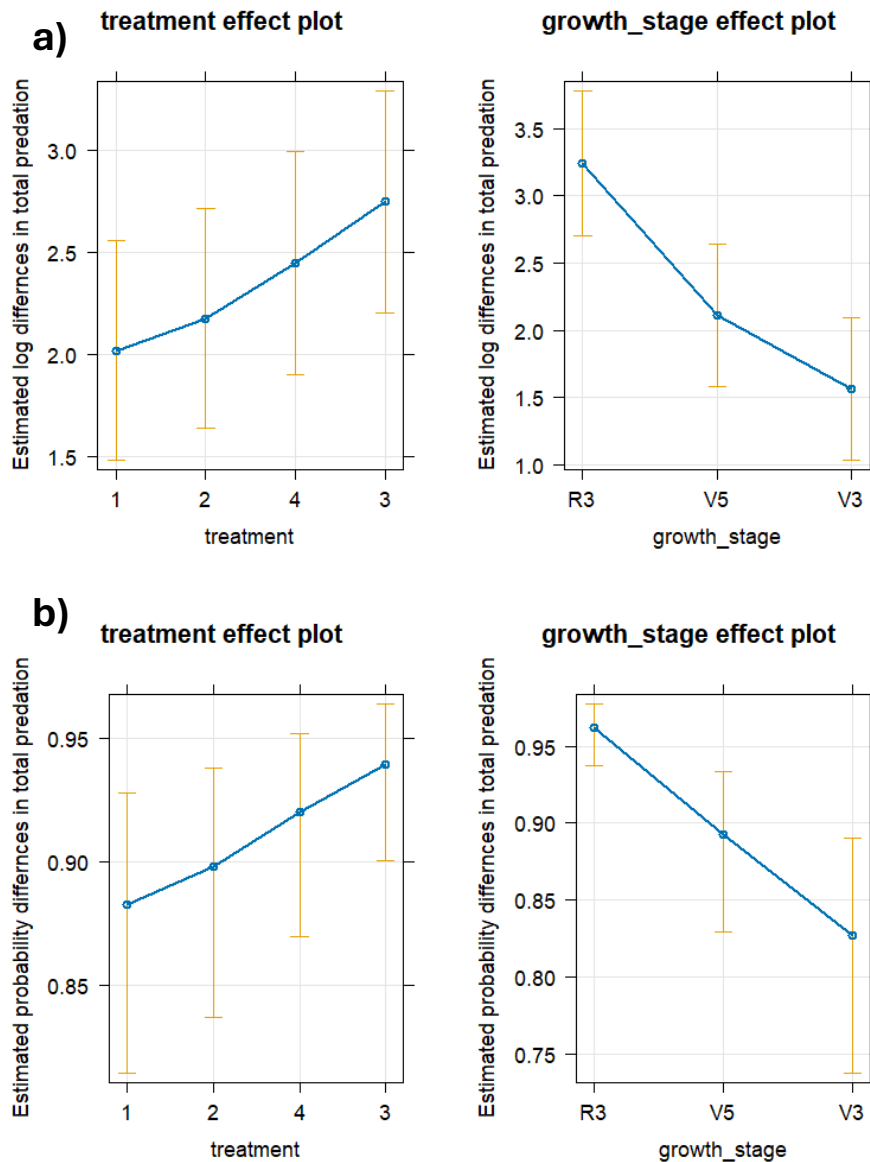


Figure 4: **a)** Effects plots of the estimated log differences in predation among treatments and growth stages. **b)** Effects plots of the estimated probability differences in predation among treatments and growth stages.

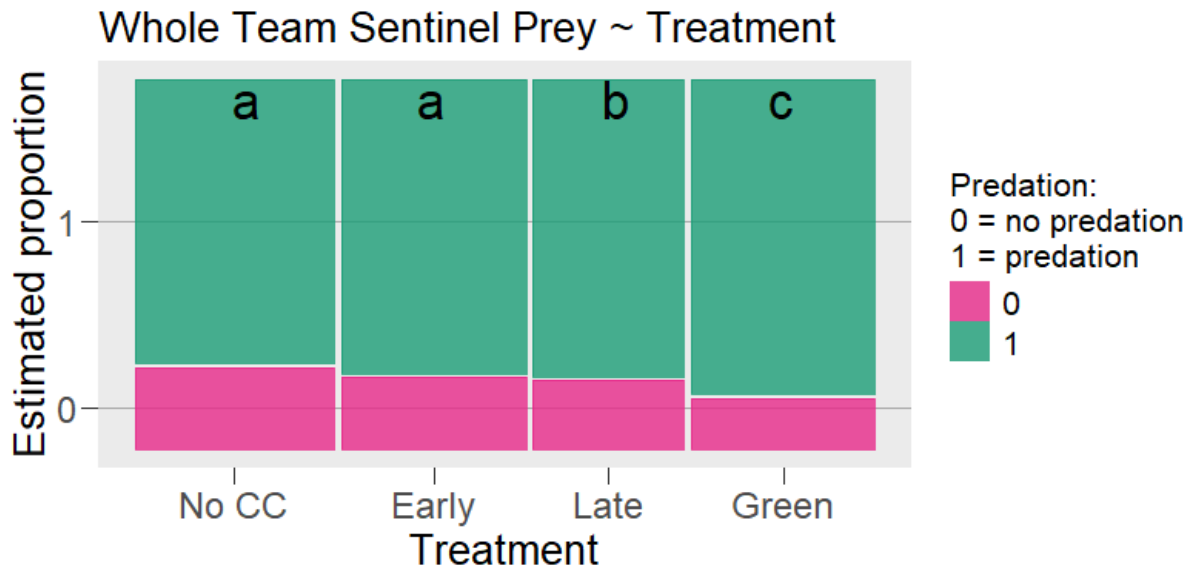


Figure 5:
Total predation binary where 1 = predation and 0 = no predation.
Predation levels differed between the no-cover and late-terminated treatment and the no-cover and planting-green treatment ($p < 0.05$).
Compact letter display denotes differences among treatments.

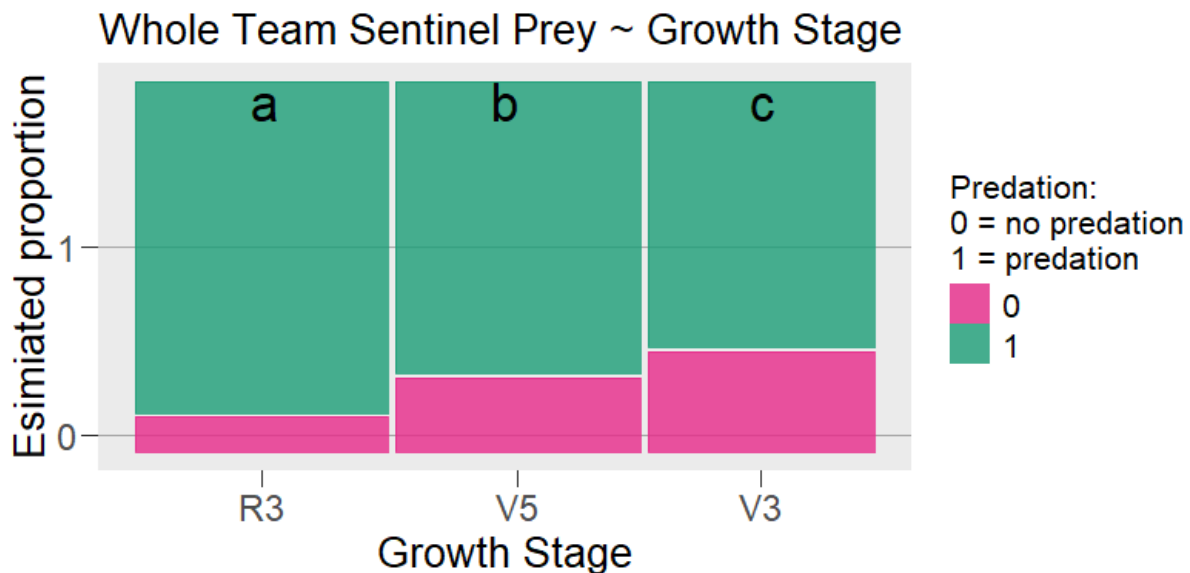


Figure 6:
Total predation binary where 1 = predation and 0 = no predation.
Predation levels differed among all growth stages ($p < 0.05$), where R3 was the highest, followed by V5, and then V3. Growth stages are ordered in reverse-chronological order.
Compact letter display denotes differences among growth stages

Tables

Treatment	Growth stage	Probability	Standard Dev.	n
No CC	V3	0.66	0.47	1098
Early terminated	V3	0.72	0.45	986
Late terminated	V3	0.74	0.44	836
Planting green	V3	0.79	0.41	982
All treatments	V3	0.72	0.45	3902
No CC	V5	0.77	0.42	1195
Early terminated	V5	0.78	0.41	1068
Late terminated	V5	0.79	0.4	871
Planting green	V5	0.84	0.36	1062
All treatments	V5	0.79	0.4	4196
No CC	R3	0.89	0.32	1146
Early terminated	R3	0.89	0.31	1149
Late terminated	R3	0.89	0.31	898
Planting green	R3	0.93	0.25	1141
All treatments	R3	0.9	0.3	4334
No CC	-	0.77	0.42	3439
Early terminated	-	0.8	0.4	3203
Late terminated	-	0.81	0.4	2605
Planting green	-	0.86	0.35	3185

Table 1: Breakdown of mean, standard deviation, and sample size for each treatment and growth stage pair. The response is on a binomial scale (1 = predation/ success, 0 = failure/ no predation). Data derived from the Mosaic package. Growth stages are order chronologically (V3, V5, R3).

Citations

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