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USDA LTAR Common Experiment measurement: Natural pest suppression

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We use this protocol and it's working

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Abstract

Pest suppression is a key ecosystem service worth billions of dollars annually, and it can vary with local management practices and landscape context. We propose to measure biocontrol services by deploying plasticine caterpillar mimics that record imprints of attacks by other organisms. Plasticine caterpillars are an easy and standardizable method indicating the attack rates on herbivorous lepidoptera larvae (Low et al. 2014, Howe et al. 2009, Haan & Landis 2023). They have been used successfully to study high-profile predation and its variation (e.g., Roslin et al. 2017). They also help identify the attacking organisms; bird beaks, arthropod mandibles, parasitoid wasp ovipositors, and small mammal teeth leave unique signatures on the dummy caterpillars.

Before start

The measurement described in this protocol for the LTAR network is dependent on acquiring extramural funding. Contact Nate Haan (**nate.haan@uky.edu**) for clarification on the ability to proceed with this protocol and whether samples will be processed centrally or at individual sites.

Overview

1 Green plasticine caterpillars (3 cm × 15 cm) will be prepared and mounted on small wooden stakes in advance at a centralized location and mailed to each LTAR Site.

2 At each site, several caterpillars will be placed in the alternative and prevailing treatments at the plot and field scales.

3 The particulars of how many caterpillars, their precise field locations, and the deployment timings will vary depending on the interests and context of each station.

4 However, we ask participating members to deploy at least 48 caterpillars across plots and fields at their site once per growing season (see Figure 1).

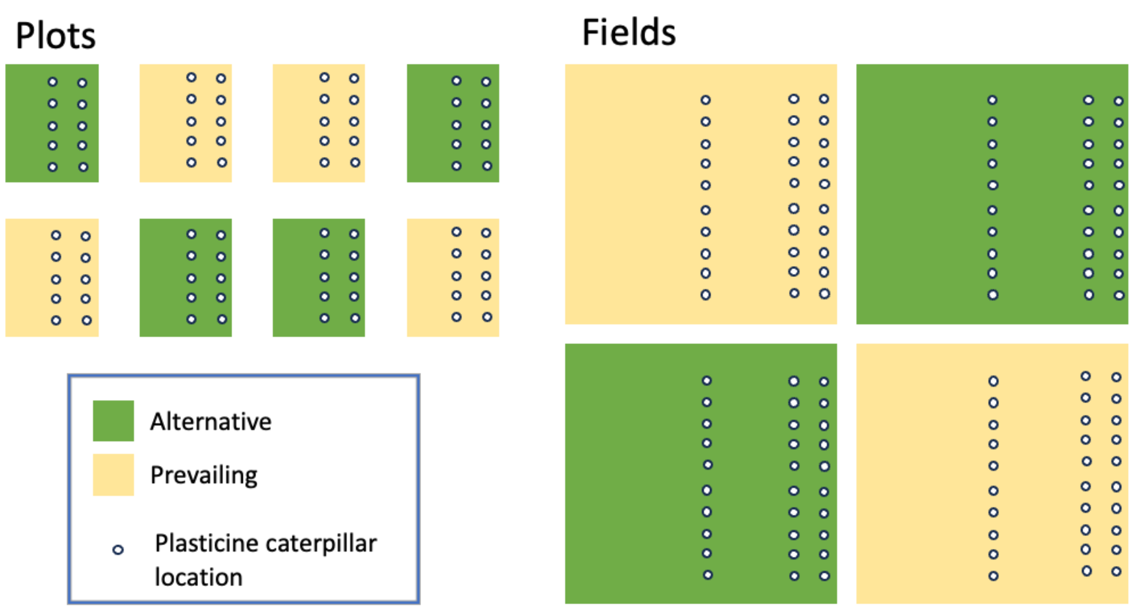

Figure 1. Hypothetical layout for deploying plasticine caterpillars at an LTAR site. Precise layouts will vary depending on site configuration and questions of interest at that site. In this example the arrangement within each plot or field allows for comparison of attack rates between field edges and interiors.

5 The caterpillars will be deployed for 2 days (48 hours), then collected and stored in protective sleeves for mailing back and completing damage assessment.

Dummy caterpillar preparation

6 We will prepare dummy caterpillars at a centralized location in advance and mail them to each participating LTAR Site (i.e., participants do not need to complete this step themselves).

7 Caterpillar production will use green Plastalina modeling clay (manufactured by Van Aken International): https://www.vanaken.com/products/4-5-lb-plastalina%E2%84%A2-non-hardening-modeling-clay-various-colors?variant=34624843120808

8 Caterpillars production will use a sugar paste extruder with a 3 mm aperture; such as the Clay Extruder Clay Gun Tool with 20 Discs available at https://a.co/d/ejat6qb.

9 The plasticine-loaded extruder will extrude a strand that will be cut into 15 mm segments using a razor blade or scalpel.

10 After examining all segments, those with imperfections will be discarded.

11 The segments will be attached to a ~15 cm bamboo kabob stake (available at most grocery stores) 3 cm from the blunt end, using a small amount of super glue.

12 Each set of caterpillars will then be mounted in foam and placed in a protective container for shipping.

Deploying and collecting dummy caterpillars in the field

13 Each LTAR Site will receive a box of ~200 mounted plasticine caterpillars, plus a few backups, for deployment at their site.

14 The number of caterpillars sent is adjustable based on site-to-site differences; however, the goal is to deploy 10 caterpillars within each plot-scale replicate and 30 within each field (i.e., for sites with four plot reps and two field reps per treatment [10 caterpillars * 2 treatments * 4 plot reps] + [30 caterpillars * 2 treatments * 2 field reps] = 200).

15 If a different number of caterpillars is desired, discuss the deployment with the central biocontrol measurement coordinator.

16 All caterpillars should be deployed +/- simultaneously within a site for 48 hours +/- 6 hours is acceptable, but attempt to minimize this variation.

Note

In general, this undertaking entails driving to a site and placing caterpillars in each plot or field, then picking them up two days later at the same time of day and in the same order so that each caterpillar receives the same amount of exposure.

17 Avoid placing the caterpillars during inclement weather, when arthropod activity could be reduced. In environments with open soil, avoid times with heavy rainfall, as the caterpillars will tend to accumulate soil particles.

18 Seasonal deployment timing will vary among sites, and managers can choose the most appropriate and interesting time of year to measure pest suppression (e.g., preceding the time of year when pest outbreaks are of the highest concern at that site).

Note

If desired, caterpillars can be deployed at the beginning, middle, and end of the growing season to capture seasonal trends.

19 The precise locations of caterpillar deployment within each plot can vary among sites depending on the locations of pre-established sampling stations or landmarks and on specific questions of interest to personnel at that site (e.g., how predation rates vary between plot edges and the plot interior or between the ground level and crop canopy).

20 Regardless of the precise location, caterpillars are easy to lose following deployment, so they should be placed in the same positions within each plot or field and marked with a pin flag or tape as applicable.

21 Place the marker ~30 cm from the caterpillar (or eye level on a plant stem directly above it) to minimize its potential effect on attack rates.

22 After deployment, take GPS coordinates for each caterpillar location. See Figure 1 for an example of how to deploy caterpillars throughout plots and fields.

23 When transferring caterpillars to the field, remove the number needed for a given plot or plot set from their shipping package and stick them in Styrofoam or a foam gardening pad to carry several at once.

Note

It is inadvisable to bring the whole package into the field, as it could be dropped or damaged.

24 Caterpillars are deployable at the ground level, the canopy level, or both, depending on relevance at a given site.

25 If caterpillars are to be deployed at ground level, insert the kabob stake into the soil until the caterpillar is ~3 cm from the soil surface.

26 Height can be adjusted upward if there is thatch or vegetation present that would touch the caterpillar.

27 Depending on the focal crop, caterpillars can be placed directly adjacent to the base of the stem, as in Figure 2a.

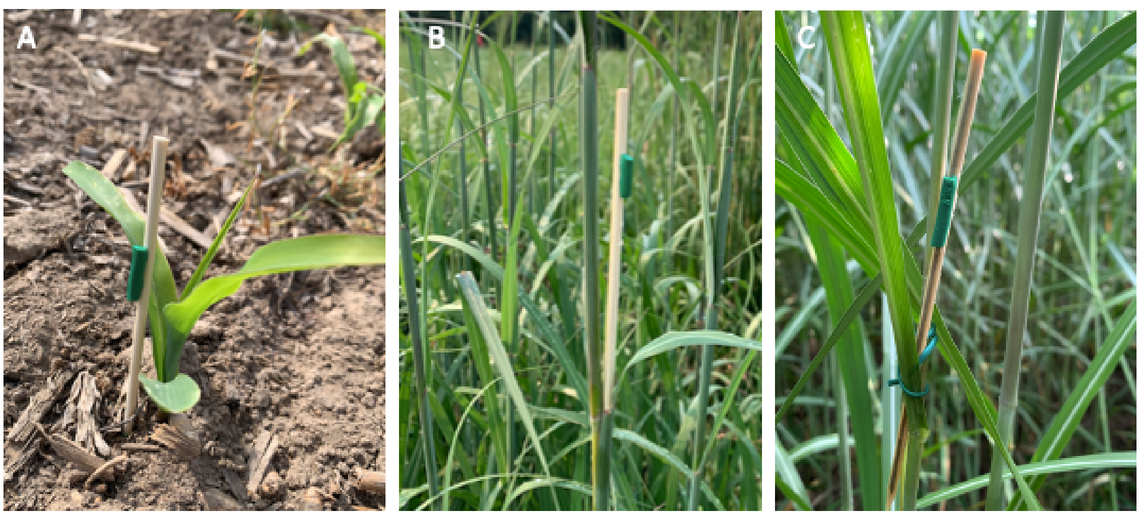

Figure 2. Methods for deploying plasticine caterpillars in soil or vegetation. A. The simplest method is to place kabob stakes directly in the soil. B. Stakes can be inserted in leaf sheaths of some grasses, including prairie grasses and corn/sorghum. C. Use masking tape or wire ties to secure the stakes if necessary.

28 If caterpillars are to be deployed in the crop canopy, the precise placement method will depend on the crop's architecture. For example, in large grasses (prairie grasses, corn, sorghum), anchor the kabob stake securely in the leaf sheath (Figure 2b).

29 In other cases, use a small amount of masking tape or a twist tie to hold the stake in place (Figure 2c). The precise method can be flexible so long as it is consistent within a site.

30 In all cases, handlers and vegetation must not touch the caterpillars. The caterpillars can record even small brushes with vegetation, clothing, and fingers, possibly making markings difficult to interpret later on.

31 When installing a caterpillar, ensure leaves will not brush against it in a breeze.

32 If a caterpillar sustains damage before deployment, replace it with a backup.

Note

Plasticine melts at approximately 150 °F, so do not keep it in closed vehicles during hot weather.

33 Label dummy caterpillars while collecting them at the end of 48:00:00 hours. 2d

34 For efficiency, write plot codes in advance on pieces of labeling tape attached to a flat surface portable in the field.

35 Each label should contain the date, plot or field treatment, replicate number, and an identifier for the station or location within the plot where the caterpillar was deployed.

36 This last piece of information will vary among sites, and metadata will need to be provided to understand these differences during analysis.

37 Once a kabob has been picked up, wrap the tape around it or skewer it to keep it attached.

38 Carry a piece of Styrofoam to transport the kabobs as you pick them up, then transfer them to the larger piece of Styrofoam they were shipped on after completing caterpillar collecting from a given plot.

39 Finally, once all caterpillars are collected and placed in their original package, they can be shipped back to the central location for evaluation.

Note

Shipping these specimens entails no overall considerations, although some individuals freeze them to avoid incidental bumps.

Evaluation, analysis

40 Each plasticine caterpillar will undergo inspection using a dissecting microscope. Damage to each caterpillar will be recorded.

41 Specifically, each caterpillar will be scored with a 0 (no marks), 1 (≤10% of caterpillar length damaged), 2 (11-50% damaged), or 3 (>50% damaged) for each of the following types of markings: small mammal teeth, bird beaks, arthropod mandibles, arthropod ovipositors, incidental contact with vegetation, and incidental contact with a human during installation.

Note

Details on differentiating the marks of different types of organisms are found in Low et al. (2014).

42 To minimize observer bias, the same individual should review all specimens.

43 Alternatively, multiple technicians can assess each caterpillar, with discrepancies between two observers averaged or resolved by a senior team member.

44 Differences in attack odds are assessable with binomial generalized linear mixed models, and differences in the community composition of the attacking organisms (i.e., types of bite marks) are detectable using multivariate techniques such as Permutational MANOVA.

Quality control

45 Double-check all plasticine caterpillars during their installment to ensure they have no accidental markings from handling.

46 Do not expose plasticine to high temperatures, as caterpillars will lose their form at 150 °F .

47 During evaluation, the number of observers will be limited, or multiple technicians will evaluate each caterpillar, with discrepancies resolved by a senior team member. Training materials may be provided to ensure all observers follow the same standards.

Archiving

48 Each site should submit metadata describing the labeling scheme for plasticine caterpillars and GPS coordinates for all deployment locations. Beyond the treatment and replicate IDs, this submission should include information about where caterpillars were deployed (at an edge or the middle of the plot/field and in the canopy or soils), the crop or land use type they were deployed in, etc.

49 All caterpillars will be photographed before disposal so that images can be checked in the future as needed (retaining original samples is usually not feasible due to space constraints).

Recommendations for data collection

50 Table 1. Summary of recommendations for measuring natural pest suppression.

A	B	C	D
Attribute	Preferred	Minimum	Comments
Spatial scale	Plot and Field	Plot	
Frequency	Annually	Every 2-3 years	Collecting data multipl e times per season is a lso encouraged if pers onnel at the site wish t o understand within-ye ar seasonal variation
Covariate metrics			Can be linked to data o n pest infestations whe n applicable on a site-b y-site basis

Protocol references

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