# Developing an Effective Chemical Treatment Protocol for Yaupon Holly

Proposal Version 1.5 (April 21, 2026)

## 1. Purpose and Management Context

This study will develop a broadcast herbicide treatment protocol to control regrowing yaupon holly (*Ilex vomitoria*) following mechanical brush management. The intended use case is large-scale management areas where Individual Plant Treatment (IPT) is operationally infeasible.

## 2. Research Objectives

The study has three primary objectives:

1. Maximize yaupon holly mortality and/or long-term suppression.
2. Minimize impacts to non-target herbaceous vegetation, including native grasses and forbs.
3. Minimize unintended impacts to overstory trees.

## 3. Herbicides Evaluated

The following herbicides were selected based on prior evidence of effectiveness in the post oak savannah ecoregion:

- Triclopyr 3 (amine formulation)
- Triclopyr 3 + Metsulfuron methyl (MSM)
- Triclopyr 4 (ester formulation)
- Invora®

# 4. Herbicide Mixes and Application Rates

| Treatment Mixes | Gallons per acre | Quarts per Acre | Precent in Mix* | Gallons per Tank of Size (gal.) | | | Quarts per Tank of Size (gal.) | | Onces per Tank of Size (gal.) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 12.5 | 30 | 60 | 30 | 60 | 30 | 60 |
| **Triclopyr 3** | | | | | | | | | | |
| Triclopyr 3 | 1.13 | 4.50 | 3.75% | | 1.13 | 2.25 | 4.50 | 9.00 | 144.00 | |
| Cide-Kick II | 0.38 | 1.50 | 1.25% | | 0.38 | 0.75 | 1.50 | 3.00 | 48.00 | 96.00 |
| Water | | | 95.00% | | 28.50 | 57.00 | | | | |
| | | | | | | | | | | |
| **Triclopyr 3 + MSM** | | | | | | | | | | |
| Triclopyr 3 | 1.13 | 4.50 | 3.75% | | 1.13 | 2.25 | 4.50 | 9.00 | 144.00 | |
| MSM | 0.01 | 0.03 | 0.03% | | 0.01 | 0.02 | 0.03 | 0.06 | 1.00 | 2.00 |
| Cide-Kick II | 0.38 | 1.50 | 1.25% | | 0.38 | 0.75 | 1.50 | 3.00 | 48.00 | 96.00 |
| Water | | | 94.97% | | 28.49 | 56.98 | | | | |
| | | | | | | | | | | |
| **Strong Triclopyr 3** | | | | | | | | | | |
| Triclopyr 3 | 1.69 | 6.75 | 5.63% | | 1.69 | 3.38 | 6.75 | 13.50 | 216.00 | |
| Cide-Kick II | 0.38 | 1.50 | 1.25% | | 0.38 | 0.75 | 1.50 | 3.00 | 48.00 | 96.00 |
| Water | | | 93.13% | | 27.94 | 55.88 | | | | |
| | | | | | | | | | | |
| **Triclopyr 4** | | | | | | | | | | |
| Triclopyr 4 | 0.83 | 3.33 | 2.78% | | 0.83 | 1.67 | 3.33 | 6.67 | 106.67 | |
| MSO | 0.42 | 1.67 | 1.39% | | 0.42 | 0.83 | 1.67 | 3.33 | 53.33 | 106.67 |
| Water | | | 95.83% | | 28.75 | 57.50 | | | | |
| | | | | | | | | | | |
| **Invora** | | | | | | | | | | |
| Invora | 0.28 | 1.13 | 0.94% | | 0.28 | 0.56 | 1.13 | 2.25 | 36.00 | 72.00 |
| MVO Plus 1 | 0.15 | 0.60 | 0.50% | | 0.15 | 0.30 | 0.60 | 1.20 | 19.20 | 38.40 |
| Water | | | 98.56% | | 29.57 | 59.14 | | | | |
| | | | | | | | | | | |
| **Triclopyr 4 + Diesel** | | | | | | | | | | |
| Triclopyr 4 | 1.25 | 5.00 | 10.00% | 1.25 | | | | | | |
| Diesel | 11.25 | | 90.00% | 11.25 | | | | | | |

*Assumes an application rate of 30 gallons of mix per acre except for the Triclopyr 4 + Diesel Mix which assums 12.5 gallons of mix per acre

# 5. Treatment Timing and Seasonal Protocols

Each herbicide will be evaluated under multiple seasonal broadcast application regimes. The following timing windows define each regime:

- **Spring** *(regime name)*: Applied May–June of the first growing season
- **Fall** *(regime name)*: Applied August–September of the first growing season
- **Split** *(regime name)*: Applied August–September of the first growing season, then again August–September of the second growing season

- **Dormant** *(regime name)*: Applied December–January of the first growing season

Herbicide assignments by regime are as follows:

- **Triclopyr 3 and Triclopyr 4** will be evaluated under the Spring, Fall, and Split regimes (Dormant regime excluded).
- **Invora®** will be evaluated under the Spring, Fall, and Dormant regimes (Split regime excluded).
- **Strong Triclopyr 3** will only be evaluated under the Fall regime.

In addition, we will include three non-broadcast comparison protocols:

1) **Individual Plant Treatment (IPT) with the Triclopyr 4 + Diesel mix:** This is a well-established method for yaupon control and typically achieves very high mortality. However, it is costly and operationally impractical for large projects (>20 acres). Application can also cause collateral damage to non-target species, particularly when using high-volume equipment. In this study, IPT will provide a benchmark "best-case" kill rate and allow us to quantify non-target impacts associated with IPT. We will apply the mix using a UTV-mounted spray tank and a handheld spray wand (lower labor than a backpack sprayer, but potentially greater non-target exposure).

2) **Shredding with a rotary cutter:** This mechanical-only option will be included as an operational alternative when herbicide use is not desired or feasible. It is not expected to produce high yaupon mortality, but it may temporarily suppress woody regrowth and promote fine-fuel development to support subsequent prescribed burning.

3) **No treatment:** Untreated plots will serve as a control to quantify background yaupon regrowth and to establish a baseline for any vegetation impacts attributable to mechanical mulching alone.

# 6. Treatment Methods (n = 16)

The 16 treatment methods are listed below.

| Group | ID | Primary Herbicide(s) | Timing Regime |
|---|---|---|---|
| 1 | **T3S** | Triclopyr 3 | Spring |
| 2 | **T4S** | Triclopyr 4 | Spring |
| 3 | **TMS** | Triclopyr 3 + MSM | Spring |
| 4 | **T3F** | Triclopyr 3 | Fall |
| 5 | **T4F** | Triclopyr 4 | Fall |

| | | | |
|---|---|---|---|
| 6 | **TMF** | Triclopyr 3 + MSM | Fall |
| 7 | **T32** | Triclopyr 3 | Split |
| 8 | **T42** | Triclopyr 4 | Split |
| 9 | **TM2** | Triclopyr 3 + MSM | Split |
| 10 | **IS** | Invora | Spring |
| 11 | **IF** | Invora | Fall |
| 12 | **ID** | Invora | Dormant |
| 13 | **IPT** | Triclopyr 4 + Diesel (IPT) | Fall |
| 14 | **C** | Control (no treatment) | |
| 15 | **ST3F** | Triclopyr 3 (High Strength) | Fall |
| 16 | **S** | Shredding only | Fall |

# 7. Experimental Design

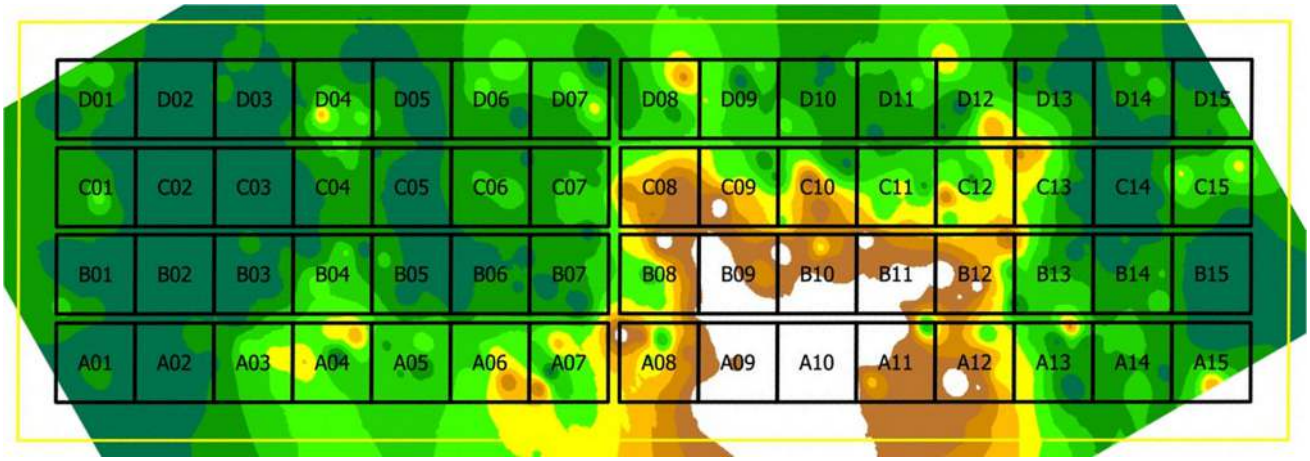
- **Design:** Randomized complete block design (RCBD). This design was selected because environmental conditions (e.g., canopy cover, soil moisture, basal area) are expected to vary spatially across the study area. By grouping plots into blocks that each contain all 15 chemical and control treatments, the RCBD accounts for this spatial heterogeneity, reducing error variance and improving the statistical power to detect treatment differences. The shredding-only treatment will be assigned in the margins of the study area as it fits. Randomization of treatment assignments within each block ensures unbiased estimates of treatment effects.
- **Treatments per block:** 16. Each block contains one plot assigned to each of the 15 treatment methods defined in Section 6. The “complete” nature of the design — meaning every treatment appears in every block — ensures that all herbicide and timing combinations can be directly compared within each replicated unit, regardless of between-block environmental variation.
- **Replications:** 4 blocks. Four replications provide adequate statistical power to detect meaningful differences in yaupon response among treatments while remaining operationally feasible within the available management area. Replication also allows blocks to be modeled as a random effect in the analysis, capturing site-level variation that is not attributable to treatment.
- **Total plots:** 64 (16 treatments × 4 blocks). This plot count reflects the full factorial combination of all treatment methods across all replications, ensuring a balanced and complete dataset for analysis.

- **Plot size:** 0.25 acres (104 ft × 104 ft) per plot. This plot size was chosen to be large enough to capture meaningful variation in yaupon cover, density, and mortality responses at a scale relevant to broadcast herbicide application, while allowing all 60 chemical and control plots to fit within the available treatment area. The square footprint simplifies layout, boundary demarcation, and the consistent application of broadcast treatments. The shredding-only treatment plots will be 30 ft by 160 ft to fit into the perimeter buffer area and as they have no internal buffers.
- **Buffers:** 12 ft between adjacent columns of plots, 20 feet between row 7 and 8 of the plots, 50 ft surrounding the entire experimental area. All buffers will be mulched at the beginning of the study and then shredded as needed to support travel within the research area. The 50 ft perimeter buffer provides a transitional zone that separates the experimental plots from unmanaged surrounding vegetation, minimizing edge effects and ensuring that observed responses reflect applied treatments rather than external influences. The shredding-only treatment plots will be fit into the perimeter buffer as it should not be impacted by edge effects. In addition, no measurements will be taken in the outer 10 feet of each plot boundary to minimize the impact of cross-contamination of plot treatments (this does not apply to the shredding-only treatment plots as they are removed from the chemical treatment plots).

The figure below shows the experimental blocks (color-coded) and the treatment method assigned to each plot for the chemical and control plots.

| | | Plot Rows | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | |
| Plot Columns | D | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 | D11 | D12 | D13 | D14 | D15 | Plot Id |
| | | 2 | 5 | 7 | 3 | 8 | 4 | 10 | 13 | 12 | 14 | 1 | 2 | 5 | 8 | 1 | Treatment Group |
| | | T4S | T4F | T32 | TMS | T42 | T3F | IS | IPT | ID | C | T3S | T4S | T4F | T42 | T3S | Treatment ID |
| | C | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | C9 | C10 | C11 | C12 | C13 | C14 | C15 | Plot Id |
| | | 13 | 4 | 9 | 10 | 11 | 15 | 14 | 10 | 7 | 4 | 3 | 10 | 7 | 11 | 12 | Treatment Group |
| | | IPT | T3F | TM2 | IS | IF | ST3F | C | IS | T32 | T3F | TMS | IS | T32 | IF | ID | Treatment ID |
| | B | B1 | B2 | B3 | B4 | B5 | B6 | B7 | B8 | B9 | B10 | B11 | B12 | B13 | B14 | B15 | Plot Id |
| | | 12 | 14 | 8 | 3 | 13 | 9 | 1 | 5 | 2 | 11 | 8 | 13 | 15 | 9 | 3 | Treatment Group |
| | | ID | C | T42 | TMS | IPT | TM2 | T3S | T4F | T4S | IF | T42 | IPT | ST3F | TM2 | TMS | Treatment ID |
| | A | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 | A9 | A10 | A11 | A12 | A13 | A14 | A15 | Plot Id |
| | | 15 | 11 | 6 | 1 | 7 | 2 | 6 | 12 | 6 | 5 | 15 | 9 | 14 | 4 | 6 | Treatment Group |
| | | ST3F | IF | TMF | T3S | T32 | T4S | TMF | ID | TMF | T4F | ST3F | TM2 | C | T3F | TMF | Treatment ID |
| | Note: A1 is southwest corner of the study area, and D15 is the northeast corner of the study area | | | | | | | | | | | | | | | | |

The figure below overlays the plot grid on a canopy-cover map (dark green = 100% cover; white = 0% cover).




## Amount of chemicals to be applied

The tables below analyze the amount of each mix component (other than water) to be applied and the number of acres it will be applied to.

**Acres treated per season with each herbicide mix**

| Treatment Method | Spring | Fall | Dormant | Fall of 2nd Growing Season | Total Acres Treated |
|---|---|---|---|---|---|
| Triclopyr 3 | 1 | 2 | 0 | 1 | 4 |
| Triclopyr 3 + MSM | 1 | 2 | 0 | 1 | 4 |
| Strong Triclopyr 3 | | 1 | 0 | 0 | 1 |
| Triclopyr 4 | 1 | 2 | 0 | 1 | 4 |
| Invora | 1 | 1 | 1 | 0 | 3 |
| Triclopyr 4 + Diesel | 0 | 1 | 0 | 0 | 1 |

**Total amount of mix component needed for the entire study (other than water)**

| Mix component | Precent in Mix* | Gallons per acre | Quarts per Acre | Ounces per Acre | Total Acres Treated | Total gallons needed | Total by Chem |
|---|---|---|---|---|---|---|---|
| | | | | | | | |
| Triclopyr 3 | 3.75% | 1.13 | 4.50 | | 8 | 9.00 | 10.69 |
| Strong Triclopyr 3 | 5.63% | 1.69 | 6.75 | | 1 | 1.69 | |
| Cide-Kick II | 1.25% | 0.38 | 1.50 | | 9 | 3.38 | 3.38 |
| MSM | 0.026% | 0.01 | | 1.00 | 4 | 0.03 | 0.03 |
| Triclopyr 4 | 2.78% | 0.83 | 3.33 | | 4 | 3.33 | 4.58 |
| MSO | 1.39% | 0.42 | 1.67 | | 4 | 1.67 | 1.67 |
| Invora | 0.94% | 0.28 | 1.13 | | 3 | 0.84 | 0.84 |
| MVO Plus 1 | 0.50% | 0.15 | 0.60 | | 3 | 0.45 | 0.45 |
| Triclopyr 4 at 10% | 10.00% | 1.25 | 5.00 | | 1 | 1.25 | |
| Diesel | 90.00% | 11.25 | 45.00 | | 1 | 11.25 | 11.25 |

# 8. Response Variables and Data Analysis

## Dependent variables

- Yaupon cover
- Yaupon density
- Yaupon mortality
- Plant species richness

## Fixed effects

- Herbicide treatment
- Application timing

## Random effects / covariates

- Block
- Canopy cover
- Basal area
- Air temperature during application
- Soil moisture during application

# 9. Vegetation and Yaupon Monitoring

Sampling will include:

- Line-intercept surveys
- Quadrat-based vegetation structure estimates
- Individual yaupon mortality tracking
- Belt transect density estimates

# 10. Drone Imagery

Drone imagery will be collected at the following times:

- Post-mulching, pre-treatment (early spring 2026)

- 2–3 months post-spring treatment (2026)
- Early spring before leaf out 2027 – this should reveal how much yaupon seems to have been killed by all treatments
- Winter/Spring 2027-2028 before leaf out – this should reveal how much yaupon has recovered from all treatments