The Impact of Environmental Factors on Invasive Vinca major Management in the Sonoran Desert

The Impact of Environmental Factors on Invasive *Vinca major* Management in the Sonoran Desert

Undergraduate Honors Thesis
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Abstract

Invasive species are one of the top global threats to biodiversity, and guidelines for context-dependent local management of invasive species is needed. *Vinca major* is a vine native to Eurasia that acts as an invasive in the Southwestern United States, including Aravaipa Canyon, an ecologically important corridor with a rare perennial stream in the Sonoran Desert. Eradication efforts for *V. major* in the canyon focus on glyphosate (Roundup) application. However, detailed guidance on the ideal environmental conditions under which to apply glyphosate to achieve maximum *V. major* die-off would be useful to land managers and is a current knowledge gap. To address this gap, a treatment experiment was conducted in the field. Plots of mature *V. major* were sprayed with glyphosate at regular intervals throughout the spring of 2022, capturing seasonal changes environmental conditions including temperature, humidity, daylight, and precipitation across the winter-summer gradient. Glyphosate had a substantial impact on *V. major* die-off, with early spring being the most effective time of application and late spring being less effective. Application date was a strong predictor of die-off and no single environmental factor was a better predictor of glyphosate efficacy. Date of application likely captures a combination of the many environmental factors that change over the course of the spring, such as temperature, humidity, soil moisture, and length of daylight. Based on these results, I recommend that spring glyphosate application on *V. major* be carried out no later than Julian date 100, or April 9th-10th.

Introduction

Invasion ecology and its application

Invasion ecology is an active and complex field, combining ecological theory and applied conservation biology. British ecologist Charles Elton is credited with founding the field in its modern incarnation with his 1958 book *The Ecology of Invasions by Animals and Plants*, in which he changed the conversation from one of isolated events of rapid population growth to one about a global pattern mostly facilitated by humans (Ricciardi & MacIsaac, 2008). Elton's ideas have since influenced the development of many differing theories around the mechanisms and ecological principles of invasion (Pearson et al, 2018; With, 2002; Hui & Richardson, 2017; Catford et al, 2009; Duncan & Williams, 2002; Callaway & Ridenour, 2004). Debate about conceptual models of ecological invasion is active, but there are also unified themes across the field. A wave of foundational literature in the late nineties underlined the connection between invasive species and global change, including climate change (Vitousek et al, 1997; Dukes & Mooney, 1999). This perspective is present in most current invasion and community ecology literature (LaTombe et al, 2021).

The overwhelmingly negative environmental impact of invasive species is also widely accepted, and has been widely studied, especially in the first decade of this century. There can be instances of invasive species being declared a positive addition to an ecosystem (Mazurczyk & Brooks, 2021), but these are not the rule. Invasive species are one of the leading threats to biodiversity, especially in certain regions (Khapugin et al, 2020; Roux et al, 2019; Smith et al, 2003). The Millennium Ecosystem Assessment (2005) considers them to be one of the five major threats to ecosystem integrity. They impact ecosystems in many different ways, including direct competition with native species, altering biogeochemical cycles, changing soil or tree canopy structure, and altering food webs (Dukes & Mooney, 2004; Ehrenfeld, 2003). As many as 42% of federally listed endangered or threatened species in the United States were primarily at risk because of invasive species in 2005 (Pimental et al, 2005). They can also act as a secondary impact of land cover change, because many invasive species primarily "invade" disturbed areas created by human development and resource extraction (Vitousek et al, 1997). Additionally, invasive species have major global economic costs associated with them (Pimental et al, 2005).

Invasion ecology is also an applied field, and much of the work within the last decade is focused on specific cases of invasion and their management. This work is often highly valuable to land managers within that particular case, but difficult to generalize to other invasive species management problems because the specific local context is so significant (Weidlich et al, 2020). The highly localized nature of invasive species "problems" (as determined by land managers and stakeholders) makes it difficult to apply the invasion ecology literature in new or understudied invasive species contexts.

Glyphosate as a method of invasive species removal

The applied side of invasion ecology- which usually means invasive species removal, or at least management- includes a variety of methods. Interestingly, Weidlich et al (2020) found in a global review that one of the strongest predictors for the method of invasive species removal was the Human Development Index (HDI) of the country where the removal took place. Chemical controls were used in about 40% of the invasive species removal cases occurring in "very high" HDI countries, including the United States, and almost none of the time in "low" and "medium" HDI countries (Weidlich et al, 2020). Of the chemical controls used, the herbicide glyphosate was by far the most common, occurring in about 40% of cases where chemical controls were used (Weidlich et al, 2020). Considering glyphosate's role as the most ubiquitous herbicide in invasive species removal, and its anticipated usage in this project, it is important to examine its functions and potential risks in the context of ecological restoration.

Glyphosate has been widely used in agriculture, landscaping, and other applications since it was introduced to the market as Roundup in 1976. It has even been referred to as a "once-in-a-century herbicide" (Duke & Powles, 2008). Part of this success is its toxicological and environmental safety and low cost, especially compared to other herbicides. Glyphosate works by being quickly and efficiently absorbed by leaf surfaces (Giesy et al, 2000). It is deactivated inside the plant, and it has long been accepted that excess glyphosate that falls into the soil sorbs tightly to soil constituents and isn't significantly taken up by plant roots (Giesy et al 2000; Duke & Powles, 2008). Other work has found that high concentrations can in fact be taken up by nontarget plant roots, but only under specific and unusual soil conditions (Cornish & Burgin, 2005). Glyphosate has a very low K_{ow}, so it does not bioaccumulate in animal fat, and is considered one of the least toxic herbicides to animals (Giesy et al, 2000; Duke & Powles, 2008). When it comes to human health, glyphosate was originally considered completely safe and non-carcinogenic (Williams et al 2000), but occupational glyphosate exposure has since been linked to Non-Hodgkin's Lymphoma (Alavanja & Bonner, 2012), and lawsuits against glyphosate manufacturers and employers over cancer diagnoses have been on the rise in the past decade (Carreiro et al, 2020).

Many of these safety assessments assume standard application quantities, but this is not necessarily the case in actual field spraying to remove invasives. Uncontrolled application methods, such as backpack and handgun sprayers (which are more commonly used than controlled methods in invasive species removal), tend to cause overspraying (Cornish & Burgin,

2005) especially when the people applying glyphosate are untrained (Carreiro et al, 2020). Any glyphosate application for invasive species removal must be done with these risks in mind. However, these risks should also not be used to discount glyphosate's utility, given that it is still the safest low-cost herbicide and has been an extremely useful tool for removing invasive species and promoting native biodiversity (Beck, 2014; Bohn et al, 2011; Carreiro et al, 2020; Love & Anderson, 2009).

Aravaipa Canyon and *Vinca major* as an invasive species

Aravaipa Canyon is a remote, steep-walled canyon in the Sonoran Desert of Arizona. It is very ecologically significant: it is one of the few places in the Sonoran Desert with perennial water flow, and therefore supports a substantial diversity of trees, herbaceous plants, birds, amphibians, insects, and mammals, as well as the most intact native fishery in the state (Hendrick & Haberstich, 2019; Turner & List, 2007). *Vinca major* (periwinkle) was introduced to the canyon in the early 20th century as an ornamental ground cover in homestead gardens. Very little literature exists on *V. major* in Southwest desert riparian ecosystems, but what does exist agrees that it is an invasive species and a threat to biodiversity (Hendrick & Haberstick, 2019; Sky Island Alliance, 2018; Pollen-Bankhead et al, 2008).

V. major's success in Aravaipa has been explained by Cushman and Gaffney's (2010) novel life forms theory, which can trace its origins to Charles Elton's invasion ecology (1958). As an assexually-reproducing vine, it has a unique life history and survival strategies in a Sonoran It reproduces vegetatively and trailing stems root whenever they touch the soil, leading to dense, monotypic stands that crowd out native plants. It reduces the amount of food available for pollinators, because it does not usually produce flowers, while consuming far more water than any of the native desert plants in this water-scarce region (Hendrick & Haberstich, 2019; Elias et al, 2016). riparian ecosystem, giving it a disproportionate ecological advantage.

Anecdotal evidence from land managers in Aravaipa Canyon supports the use of repeated glyphosate applications to remove *V. major* mats and restore native vegetation in the canyon. However, greater documentation and analysis of this method to guide best practices would be directly useful to land managers in Aravaipa Canyon and similar Southwest desert riparian ecosystems. Given the high material and labor costs of herbicide spraying (Sky Island Alliance, 2018), and herbicide's potential environmental and human health concerns (Cornish & Burgin, 2005; Alavanja & Bonner, 2012; Carreiro et al, 2020), applying herbicide under ideal conditions may help increase the overall chances for success in eradicating *V. major* completely while minimizing financial, environmental, and human health impacts.

This project aims to build on the institutional knowledge gained during past invasive *V. major* removal efforts in The Nature Conservancy's Aravaipa Canyon Preserve, collecting and analyzing environmental data to identify the best environmental conditions for herbicide application and inform future *V. major* removal efforts. If cooler temperatures during spraying are found to be a statistically significant predictor of better *V. major* die-off, for example, land managers could use that information to make more effective use of limited time and money for removal efforts. Conversely, if environmental factors are not found to be significantly related to die-off, not trying to take those variables into account unnecessarily would also potentially benefit removal efforts by freeing them to take other, more pressing variables into account.

Methods

Test plot structure

Thirty-four 2-by-2 meter square treatment plots were created within mats of well-established *V. major*. Every two weeks, four plots were sprayed with a mixture of glyphosate, surfactant, penetrant, and water conditioning additives that has been used and adjusted for several years by TNC's Preserve Steward. Each plot was sprayed once, and the order in which they were sprayed was randomly generated. Biweekly spraying continued from late February to late May, for eight total spray dates, and environmental data was collected at each date. In the center of each 4-meter square plot, an inner 1-by-1 meter square plot was set up to define the die-off assessment boundary, in order to avoid assessing *V. major* at the plot edge that may have been sprayed unevenly (Figure 1). Two plots were left unsprayed as controls.

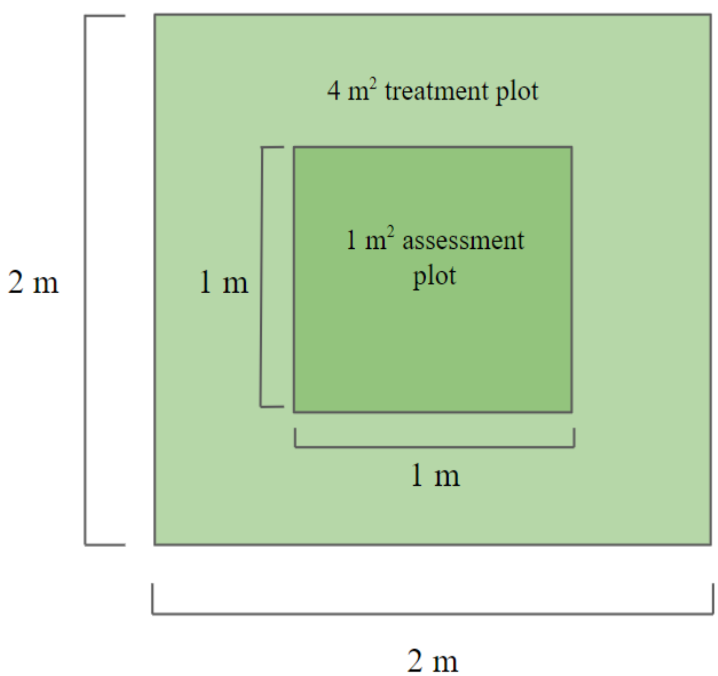

Figure 1: Treatment plot layout. The entire 4 m^2 plot is treated with glyphosate, while only the inner 1 m^2 is assessed.

Environmental data collection

Temperature, relative humidity, soil moisture, and time were recorded each week at the time of glyphosate spraying. Soil moisture was read from two soil moisture probes that were already in place in Aravaipa, and relative humidity was collected using a hand-held weather meter device. Additionally, total hours of daylight, hours of daylight remaining, and precipitation history were found after the fact by comparing the time to length-of-daylight information online and to the Preserve's detailed precipitation log. The amount of direct sunlight that a plot receives was measured using a densiometer to get an estimation of canopy cover at the beginning of the study period.

Quantifying *V. major* die-off

Baseline *V. major* measurements were established in February 2022, before glyphosate application. Two methods were used to measure die-off. Percent coverage was assessed visually, with consensus by at least two observers. Photos were also taken directly above the 1-by-1 square meter data collection plots to record percent cover. Growth stage was also assessed, using a 1-through-6 scale, for each quadrant of the assessment plot, with a 1 being total die-off and a 6 indicating a fully healthy and mature vine (see Appendix A for complete scoring guide). Growth stage of the four quadrants was then averaged to quantify the growth stage for each plot. Percent coverage and growth stage of each plot post-spraying were then assessed in July 2022, at least six weeks after the last spray date to allow the plants to die off to their maximum potential.

Statistical Analysis

The assumptions for ANOVA were not met, so a nonparametric Kruskal-Wallis test was used to test difference between treatments. In this case, the nine treatments were the nine different spray dates, plus the unsprayed control plots. The environmental factors and die-off metrics were each individually compared with a simple linear regression, and the resulting p-value matrix was used to compare strength of association. A loess regression was also included for visual comparison (see Appendix B).

Results

Spray Date and Die-off

The treatment plots showed substantially more die-off in all plots by both metrics than the control plots, although the control plots did experience slight die-off compared to their initial coverage in early February (100% coverage to 77.50% coverage across control plots, and growth stage 6 to 4.5). Among treatment plots, *V. major* die-off decreased with later spray dates, by both the percent coverage and growth stage metrics (Figure 2).

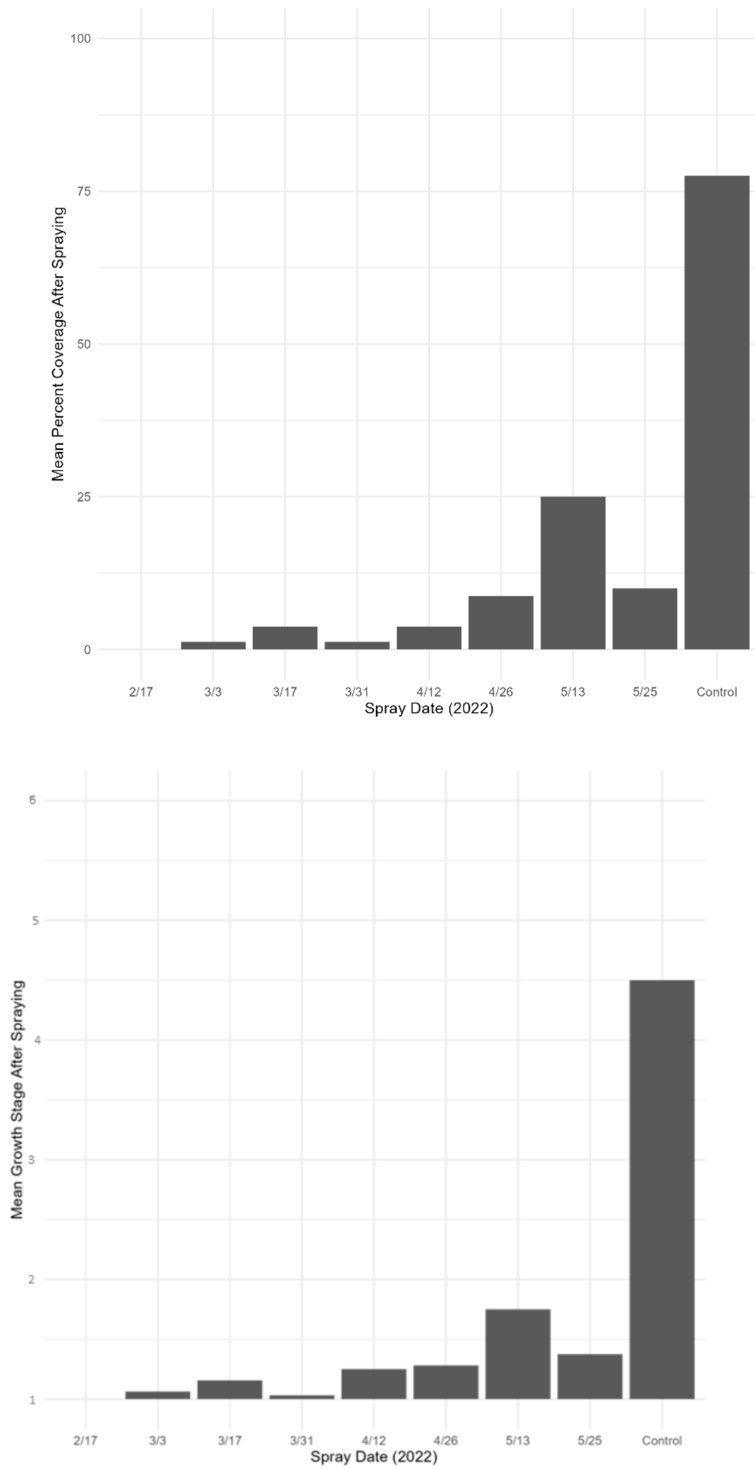

Figure 2: *V. major* die-off measured by percent coverage (top) and growth stage (bottom), by spray date.

A nonparametric Kruskal-Wallis test indicated a significant difference in plot die-off across spray date groups (p = 0.0036 for percent coverage and p = 0.0053 for growth stage). Removing the control plots raised the p-value slightly, but still indicated a significant difference between groups (p = 0.0075 and p = 0.0113).

Spray date can also be viewed as a continuous variable, using the Julian (January 1st = 1, December 31st = 365) date when spraying occurred. Spray date had a highly significant (p = 0.0002 and p = 0.0002) relationship with die-off, using a linear model (Figure 3).

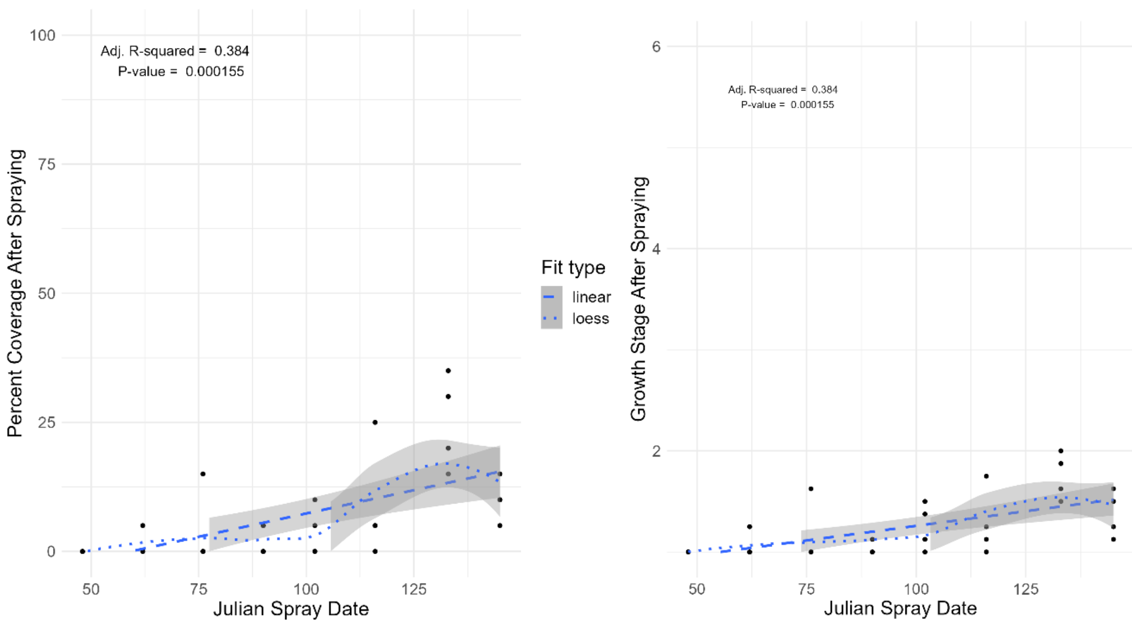

Figure 3: Spray date (Julian days) and *V. major* die-off, using percent coverage (left) and growth stage (right) metrics.

Spray Date	Average post-spraying percent coverage	Average post-spraying growth stage
2/17	0.00	1.00
3/3	1.25	1.06
3/17	3.75	1.16
3/31	1.25	1.03
4/12	3.75	1.25
4/26	8.75	1.28
5/13	25.00	1.75
5/25	10.00	1.38
Control	77.50	4.50

Table 1: Average die-off by spray date.

Environmental Variables and Die-off

The environmental variables of interest varied over the course of the study period (Table 2). Die-off had a significant linear relationship with most of the environmental variables measured, including high and low temperature, relative humidity, days since precipitation, and length of daylight. However, no environmental variable had a stronger relationship with either die-off metric than it did with Julian spray date (Table 3).

	Daily high temp. (°F)	Daily low temp. (°F)	Relative humidity	Hours of daylight	Soil moisture (probe 1)	Soil moisture (probe 2)	Days since last precip.	Accumulation (in) during last precip. event
Mean	84.63	37.75	25.79	12.67	0.02	-0.15	22.75	0.31
High	101	50	42.5	14.07	0.07	0.08	58	0.42
Low	62	22	10.9	11.1	-0.08	-0.35	1	0.05
St.Dev	11.04	7.82	11.59	0.99	0.05	0.15	19.25	0.11

Table 2: Summary statistics of environmental variables recorded at each of the 8 treatment dates.

Precipitation history variables (days since last three precipitation events and amount of rain in each event) were not ultimately useful, because there were only four total precipitation events during the study period, with the last one of the study period occurring on March 28th. This meant that for the plots that were sprayed after March 28th (62.5% of the total plots), the precipitation variables were functions of Julian date. Even for the plots sprayed before March 28th, the limited number of precipitation events caused precipitation variables to be too closely tied to Julian date to be statistically separated.

	Julian Spray Date	Percent Coverage	Growth Stage
Week Number	NA	0.0003	0.0002
Julian Spray Date	NA	0.0002	0.0002
High Temperature	2.79E-10	0.004	0.004
Low Temperature	0.001	0.007	0.035
Relative Humidity	0.0005	0.001	0.004
Daylight Length	0	0.0002	0.0001
Days Since Most Recent Precip	1.41E-11	0.0001	0.0001
Most Recent Precip Accumulation	0.184	0.580	0.471
Soil Moisture Probe 1	6.77E-09	0.010	0.006
Soil Moisture Probe 2	1.47E-08	0.015	0.013

Table 3: p-values between Julian spray date, die-off metrics (percent coverage and growth stage), and environmental factors of interest.

Early vs. Late Spring

Visual examination of die-off and Julian spray date, especially the loess model (Figure 3), suggested an inflection point around Julian day 100. To test whether there was a significant difference between die-off in plots treated before and after Julian day 100, the data were split into two groups: early spring (before day 100) and late spring (after day 100). The data did not meet the variance homogeneity assumption for a T-test (F-test $p = 0.0004$ for percent coverage and $p = 0.0201$ for growth stage), so a Wilcoxon ranked sum test was used. Both metrics were found to be significantly different between plots sprayed before day 100 and plots sprayed afterward ($p = 0.0004$ for percent coverage and $p = 0.0002$ for growth stage).

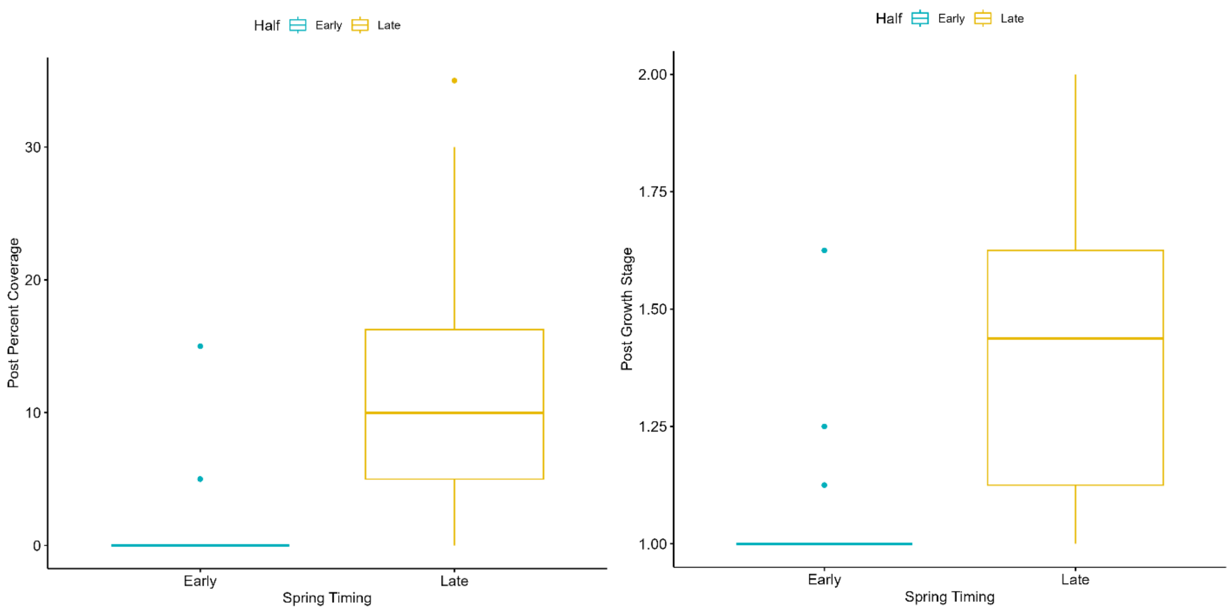

Figure 4: Distribution of *V. major* die-off in early spring (before day 100) and late spring (after day 100), quantified by percent coverage (left) and growth stage (right).

Discussion

Potential Mechanisms

The data suggest that glyphosate efficacy is not clearly driven by any particular one of the environmental variables tested here. Instead, my results suggest that Julian spray date, which is linked to several environmental factors that change over the winter-to-summer gradient, was the best predictor of glyphosate efficacy overall. This work focused on management outcomes rather than mechanisms, and therefore I cannot make any more nuanced conclusions about the mechanisms that might be at play, but there are potential mechanisms found in the literature that may help explain these findings.

Glyphosate kills plants by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase, which causes shikimate to accumulate in the cytoplasm and prevents cells from producing crucial aromatic amino acids (Schonbrunn et al., 2001). Some glyphosate can be translocated out of the cytosol, where it has access to the plastid organelles where 5-enolpyruvylshikimate-3-phosphate synthesis takes place, and into the vacuole. This process sequesters the herbicide behind the vacuole membrane, where it can no longer contribute to cell death (Tan et al, 2019). Vacuole sequestration has been found to be a mechanism of glyphosate resistance in horseweed (*Conyza canadensis)*, with temperature at the time of spraying a significant predictor of the rate of sequestration: the colder the temperature, the less glyphosate that was sequestered in vacuoles, and the more effective the glyphosate (d'Avignon & Ge, 2018; Ge et al., 2011). This effect has been found in both temperature-controlled greenhouse conditions and a field trial, with the field trial finding greatest herbicide effectiveness on cool, sunny days (d'Avignon & Ge, 2018). If glyphosate vacuole sequestration also occurs in *V. major*, with a similar rate relationship to temperature, this would help explain why glyphosate removes *V. major* more effectively earlier in the spring, during colder temperatures.

Cuticular wax, the protective lipophilic outer film on terrestrial plant leaves, is known to change thickness and/or chemical composition in response to heat stress in many plants (Liu et al., 2019; Alfarhan et al., 2020; Riederer & Schreiber, 2001). These changes help plants conserve water and thermoregulate. Such heat-related cuticular wax changes may also reduce glyphosate absorption into plant leaves, even when the herbicide is formulated with surfactants (Ou, Stahlman, & Jugulam, 2016), because they prevent both water loss and herbicide entry. Slow shifts in wax composition on the surface of *V. major* leaves over the course of the spring, as heat increases and relative humidity decreases, may reduce the amount of glyphosate that is absorbed by leaf surfaces and therefore available to cause damage within cells. Humidity can also impact how much herbicide moves through the cuticle, regardless of the wax's current temperature-related chemical composition, because herbicide droplets dry on the surface of the leaf more quickly before all the active ingredient dissolved in the droplet can be absorbed (Ramsey, Stephenson, & Hall, 2005).

Management Implications

The first clear result is that glyphosate spraying is far more effective at killing off *V. major* than no treatment at all. When it comes to environmental conditions at the time of spraying, however, the data do not suggest that any one particular environmental factor is solely predictive of die-off. Therefore no target spraying conditions (such as temperature, precipitation history, humidity, etc.) are recommended. Instead, I recommend that spring glyphosate spraying for *V. major* removal occur in an early-spring window of time. Using a date window to guide timing is likely the most effective way to ensure that spraying occurs during the time most conducive to die-off, in early spring. A target window of dates rather than environmental conditions also has the practical advantage of being a much more low-maintenance strategy: it doesn't require additional monitoring, allows for much easier advance scheduling, and is therefore more likely to be useful to land managers who are balancing many other concerns and responsibilities.

When it comes to selecting an ideal date or range of dates, there are several potential ways to interpret the data. The linear model (see fig. JSD die-off) would suggest that the earlier spraying occurs, the better (within the study period, as this study does not include data before mid-February). The loess model displays local trends in the data, and shows a relatively flat line in the early spring, which begins to climb around Julian day 100 (April 10^{th}). A Wilcoxon ranked sum test confirms a significant ($p = 0.0004$ for percent coverage and $p = 0.0002$ for growth stage) difference between die-off in plots sprayed before and after day 100. Based on this model, *V. major* glyphosate application between Julian day 48, or February 17th (the beginning of the study period) and Julian day 100, or April 10th, is recommended.

One important consideration is the interannual variation in the timing of change in environmental factors like temperature and precipitation. The months in the study period (February through May 2022) experienced slightly more extreme average daily high and low temperatures than the previous five- and twenty-year periods (Table 3), but were generally in line with past years' averages. Spring 2022 also experienced less precipitation than recent years, including no precipitation at all in April and May. These weather patterns also occur in the context of climate change, which is causing the Southwest to get hotter and drier (Gonzalez et al., 2018). The region has been in a prolonged drought since the year 2000, which may or may not continue (Williams, Cook, & Smerdon, 2022). The temperature and precipitation patterns of the study period were not substantially different from the area's "typical" recent climate (though certainly drier than usual) and were in line with predicted climate change trends. Therefore, it is reasonable to apply lessons from spring 2022 to other years. However, the fact that the study covers only one year and there is always some year-to-year variation means that the ideal spraying window will likely also vary to some degree. Therefore, we recommend avoiding spraying very close to April 10th, and instead putting a buffer in place to account for variation, such as spraying by the end of March. Data from multiple years would be needed to make a recommendation on the length of this buffer period, but land managers should use their own understanding.

		February	April	May
Monthly Max Mean Temperature	2022	67.3	86.6	95.8
	prev. 5 years:	66.3	85.06	94.33
	prev. 20 years:	66.14	82.49	91.09
Monthly Min Max Temperature	2022	28.9	44.9	52.5
	prev. 5 years:	33.9	46.96	54.4
	prev. 20 years:	33.54	46.22	53.96
Total Monthly Precipitation	2022	0.01	0	0
	prev. 5 years:	0.71	0.04	0.02
	prev. 20 years:	0.6	0.19	0.09

Table 3: Safford Agricultural Center (45 miles from study site and similar elevation) monthly mean maximum temperature, minimum temperature, and precipitation accumulation for February - May in study year, average of previous five years, and average of previous twenty years (National Weather Service, 2023).

Limitations & Recommendations for Future Research

As discussed above, the data in this study are from one year only, and cannot account for natural variation in the ways that environmental conditions shift over the course of the spring. Multiple years of data would help account for variation in environmental conditions when determining an ideal glyphosate application window. Additionally, while this study determined the end of an ideal spraying window (i.e. the latest in the spring that glyphosate should be applied for best results), the data did not capture its hypothetical start. The *V. major* that was sprayed earliest (in mid-February) died off the most, suggesting that the ideal spraying window may extend earlier in the winter. However, the data here are not able to say whether that is the case, or when the beginning of that window might be. A similar study with spraying dates started in January or even December may allow a start date of an ideal glyphosate application window to be determined, as this study was not able to do so. The most comprehensive approach would be to conduct a continuous year-round study. This would allow the start and end points of the ideal spraying period for maximum glyphosate efficacy to be identified, or potentially multiple periods throughout the year. Measuring the impact of precipitation on glyphosate efficacy would be especially useful in building on this study, because this study period received so few rain events that strong conclusions about its role in *V. major* die-off could not be made.

Conclusion

Timing of spring glyphosate application on *V. major* in a Sonoran Desert riparian zone has a strong impact on herbicide efficacy. The environmental factors included here (daily high and low temperature, length of daylight, relative humidity, days since precipitation and amount of precipitation, and soil moisture) at the time of glyphosate spraying were almost all significantly correlated with die-off, but the best single predictor was spray date. A likely explanation is that spray date represents a combination of the environmental factors included here as well as others that change over the course of the spring, and it is these factors in combination that ultimately impact glyphosate efficacy. The date effect on glyphosate efficacy does not appear to be linear, but instead *V. major* die-off begins to decrease after around Julian date 100 (April 10th) until the end of the study period. This finding is useful for Sonoran Desert riparian land management because it provides straightforward guidance for timing glyphosate application to maximize invasive species eradication success and reduce the amount of herbicide and labor required. April 10th is a reasonable recommendation based on this data from the spring of 2022, but year-to-year weather variation would be expected to cause this date to shift in other years. Spraying well in advance of April 10th is therefore recommended to account for this variation, and additional data are necessary to establish a more conservative "spray-by" date that takes year-to-year weather variation into account. Additional data from earlier in the winter, or even all year, would allow a start date of the ideal spraying window to be determined as well, because the current data do not extend early enough in the year (before mid-February) to capture it. Further research is also required to fully determine the impact of precipitation history on glyphosate efficacy due to the low amount of precipitation events during the study period. Overall, this work presents management guidance for an under-studied invasive species issue as well as opportunities for additional research.

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Appendix A: *Vinca* Growth Stage Scoring Guide

Developed by Aaron Mrotek

Vinca 6: Robust and very healthy

Vinca is dark green, with shiny leaves. Has stems greater than (1) foot. Robust evidence of rhizomes and actively spreading into adjacent areas. Leaves are wide and rounded at the tips. Usually part of large, dense mat of vinca. Flowering vinca will almost always be in this category.

Vinca 5: Healthy

Vinca is dark green, with shiny leaves. Has stems greater than one (1) foot. Evidence of rhizomes. Leaves are wide and rounded at the tips. Evidence of rhizomes and potential to spread into adjacent areas.

Vinca 4: Moderately healthy

Entire plant is alive; no brown, dead plant matter. May have evidence of new sprouts. Yellow-green leaves are present, but minimal. Has stems at least one (1) foot in length. Leaves are mostly wide and rounded at the tips.

Vinca 3: Weakened

Mostly green of some hue. Green is not quite as dark as healthy plants; may appear yellow-green. May have some brown, dead plant matter. Length of primary stems are not more than approximately one (1) foot. Leaves have a less shiny surface than healthy. Leaves are thinner and may be pointy at the tips.

Vinca 2: Damaged and dying

Clearly unhealthy. Plant has green, living tissue, but majority brown, dead tissue. Stems may still be green with brown leave Often light-green colored. Not many leaves per stem. No signs of new growth. Leaves are thin and pointy at the tips.

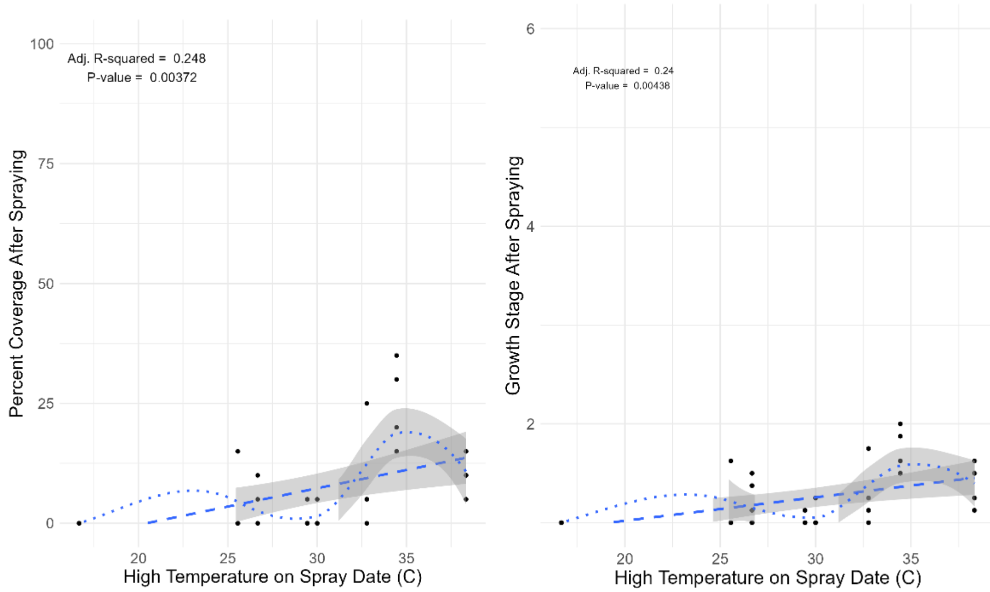
Vinca 1: Dead

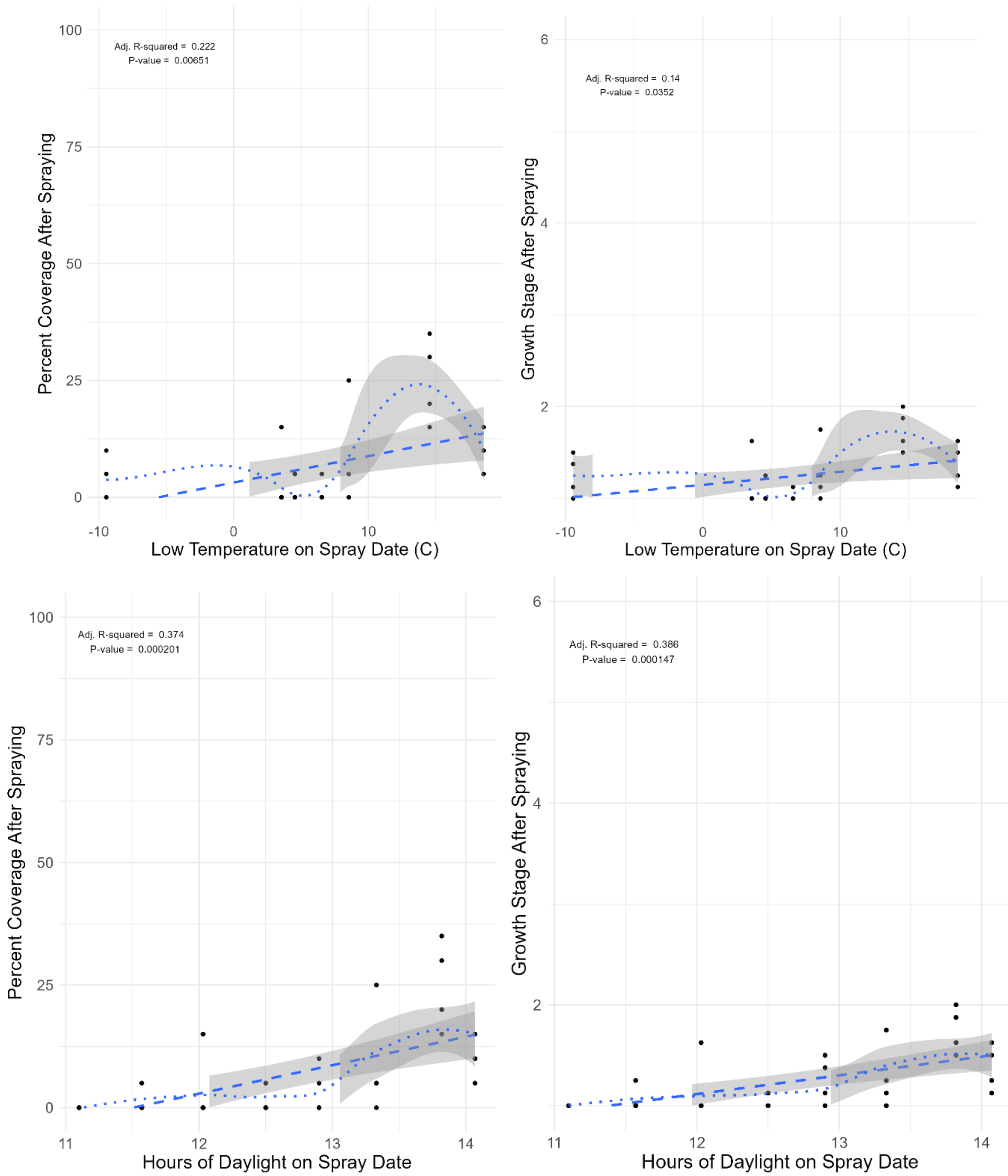
No signs of life on stem. Less than 5 percent of the plant has green on it.

Photos by Aaron Mrotek.

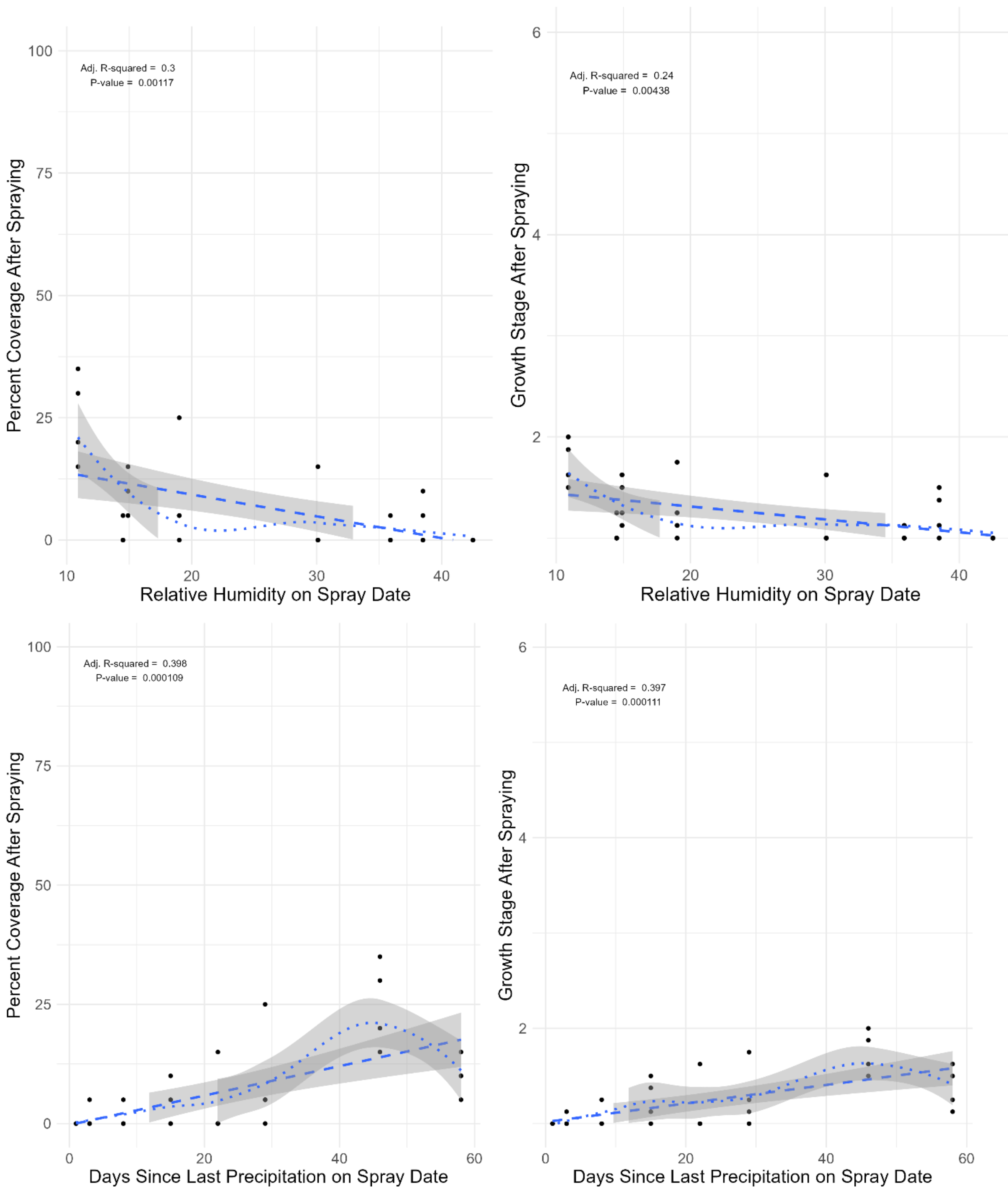
Appendix B: Environmental Variables and Die-Off Plots

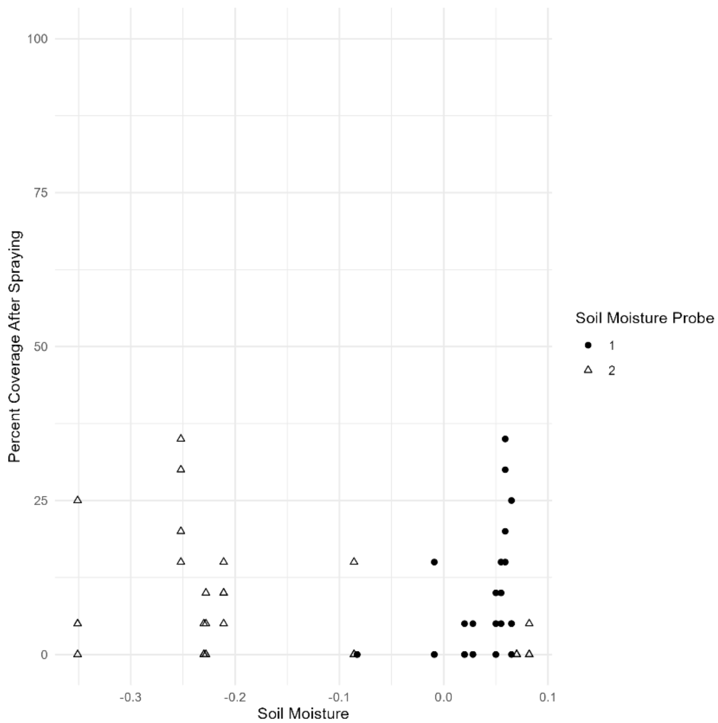
Dashed lines represent a linear model, and dotted lines represent a loess model.



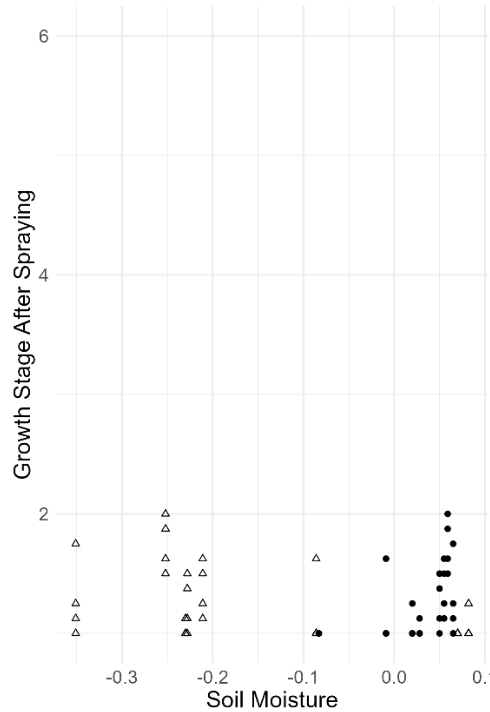


Adj. R-squared = 0.222
P-value = 0.00651
Percent Coverage After Spraying
Low Temperature on Spray Date (C)
Adj. R-squared = 0.14
P-value = 0.0352
Growth Stage After Spraying
Low Temperature on Spray Date (C)
Adj. R-squared = 0.374
P-value = 0.000201
Percent Coverage After Spraying
Hours of Daylight on Spray Date
Adj. R-squared = 0.386
P-value = 0.000147
Growth Stage After Spraying
Hours of Daylight on Spray Date


Adj. R-squared = 0.3
P-value = 0.00117
Percent Coverage After Spraying
Relative Humidity on Spray Date
Adj. R-squared = 0.24
P-value = 0.00438
Growth Stage After Spraying
Relative Humidity on Spray Date
Adj. R-squared = 0.398
P-value = 0.000109
Percent Coverage After Spraying
Days Since Last Precipitation on Spray Date
Adj. R-squared = 0.397
P-value = 0.000111
Growth Stage After Spraying
Days Since Last Precipitation on Spray Date



Percent Coverage After Spraying
100
75
50
25
0
-0.3
-0.2
-0.1
0.0
0.1
Soil Moisture
Soil Moisture Probe
1
2



Growth Stage After Spraying
6
4
2
-0.3
-0.2
-0.1
0.0
0.1
Soil Moisture