TOOLS FOR IMPROVED MANAGEMENT OF BUFFELGRASS IN THE SONORAN DESERT

by

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As members of the Dissertation Committee, we certify that we have read the dissertation prepared by Travis Bean, titled Tools for improved management of buffelgrass in the Sonoran Desert and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

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ABSTRACT

Buffelgrass is a perennial C_4 bunchgrass that is invasive in many subtropical regions worldwide. Buffelgrass has a rapid invasion rate, a tendency to displace native vegetation, and presents a fire risk to native plant communities, adjacent developed areas and their associated infrastructure. Mechanical control is often impractical and unable to keep pace with its spread. Chemical control has offered the most promise for successful and cost-effective management on a regional scale. The predominant herbicide used to control buffelgrass is glyphosate, which requires active vegetative growth when applied for optimum uptake and translocation to meristematic tissue. The timing and duration of active growth is difficult to predict. In this dissertation I addressed three related topics to improve effectiveness of buffelgrass management in the Sonoran Desert.

First, we used digital time-lapse photography and site specific weather data to predict the timing and length of future active growth during the summer based on day of year and antecedent weather at three sites in the Sonoran Desert near Tucson, AZ that were representative of habitats currently infested by buffelgrass in the region. They included a level (water-accumulating) basin floor site with a deep soil and that received supplemental moisture as precipitation runoff from adjacent areas, a slope site receiving relatively higher seasonal precipitation, and a more xeric slope site. Our predictions allow for glyphosate herbicide application efforts to be directed to time periods and sites where plants are most susceptible. We evaluated relationships among temperature, precipitation, relative humidity, and plant greenness (a proxy for active plant growth). We collected data over the 2012 and 2013 summer growing seasons (June-October), using the 2012 data to train predictive multiple regression models and 2013 data to test model prediction

accuracy. Model effectiveness was gauged by prediction agreement with observations at a threshold of 60% greenness, which represents the phenological stage where buffelgrass becomes susceptible to mortality with glyphosate application. Accuracy of models constructed using data from all sites included differed only minimally from site-specific models. Each site had different patterns of model accuracy, as measured by the probability that the predicted and observed greenness would both be either above or below 60% for a given date. We were able to correctly predict greenness above or below the 60% threshold at 81 to 95% for the basin floor site up to 28 days into the future, at 61-88% for the slope site receiving higher precipitation up to 14 days into the future, and at 62-82% for the more xeric slope site at 0-28 days into the future.

Second, we evaluated the effects of different rates of two herbicides (imazapic and clethodim), alone or in combination with different rates of glyphosate, for pre- (imazapic only) and postemergence control of buffelgrass. We also evaluated growing- and dormant-season (summer and winter, respectively) application of imazapyr for pre- and postemergence control of buffelgrass. We conducted a series of replicated field experiments from 2010 to 2013 at an undisturbed wildland site and a former agricultural field near Tucson, AZ. We used CO_2 pressurized sprayers to apply herbicides at known pressures, speeds, and carrier rates to ensure accurate broadcast application rates. Our results indicate that when applied alone, glyphosate rates of 2.52 kg acid equivalent (ae) ha^{-1} are needed to kill mature plants in a single application. Lower rates of glyphosate were effective when combined with imazapic. Imazapic did not kill mature buffelgrass plants even at the highest label rate, although this rate (0.21 kg ae ha^{-1}) did suppress shoot growth at 36 months after treatment (MAT). Clethodim did not have any effect on buffelgrass, even at the highest label rate. At one of two sites, imazapyr killed mature buffelgrass plants 6 MAT when applied during the dormant season at 0.56 kg ae ha^{-1}, or 12 MAT when applied during the growing season at 1.12 kg ae ha^{-1}. Imazapyr provided effective preemergence control of buffelgrass at all rates 6 and 12 MAT.

Finally, we evaluated the vegetation monitoring results from a helicopter broadcast herbicide application trial conducted in the Tucson Mountains. Helicopter application offers the potential to reach buffelgrass populations that are essentially inaccessible on foot and to control a larger infested area at a lower cost per area than ground-based efforts. The goal of our evaluation of the trial was to evaluate the effectiveness of helicopter broadcast herbicide application of glyphosate for buffelgrass control and also the potential for effects on native non-target vegetation. In summer 2010, federal agencies and two local jurisdictions conducted an aerial application field trial in the southern Tucson Mountains southwest of Tucson, AZ. We subsequently investigated the efficacy of the helicopter broadcast applied glyphosate for buffelgrass control and the concomitant injury these applications caused to native non-target vegetation. Two rates of glyphosate, 1.13 and 2.23 kg ae ha^{-1}, were each sprayed in carrier volumes of 46.9 and 93.8 L ha^{-1} of water for a total of four treatments. However, a related study (Thistle et al., 2014) found that the rates of glyphosate deposited on the ground were much less than what was released from the helicopter (26-76% of released) due to the height of spray release, droplet size and environmental factors. Sites were chosen to contain both buffelgrass, which was monitored for plant health one-year following glyphosate application, and representative non-target vegetation; over 1,600 individual non-target plants were marked and monitored for at least two years after the glyphosate applications. Buffelgrass was also monitored under paloverdes above 1.5 m in height and in plant interspaces to evaluate canopy effects. Glyphosate deposition decreased with increasing time of day, which was likely due to atmostpheric instability (Thistle et al. 2014). These times also coincided with all 46.9 L ha^{-1} carrier rate treatments, potentially confounding carrier rate as a model factor. Buffelgrass control increased with increasing glyphosate deposition rate and glyphosate deposition rate had a significant interaction with plant location in relation to paloverde canopies. Saguaros and other cacti were not affected by any of the treatments in year one or two post glyphosate application. Generally, response of non-target vegetation followed a trend where shrubs of increasing height incurred less damage (measured as change in plant greenness) from herbicide treatments than did smaller life forms. Brittlebush, limberbush, ocotillo, little leaf paloverde, triangle leaf bursage, and wolfberry were the only non-target plants in the study to experience damage from any of the treatments. Buffelgrass had a higher rate of increasing damage per kg ae ha^{-1} glyphosate deposition than non-target vegetation except limberbush. Buffelgrass injury was consistent with ground-based applications when deposited rate of glyphosate is considered. Although damaged, buffelgrass was not killed at rates applied in this study. The protocols used in this study need to be modified to kill buffelgrass plants in a single application. Although raising glyphosate rates may be required, as a first step helicopter applications should be made early in the morning before thermal updrafts on steep slopes disperse spray droplets. Larger spray droplets and anti-evaporant adjuvants may also help more glyphosate reach the ground. This may result in greater injury or possible mortality of certain non-target species if present in buffelgrass stands. Finally, non-target damage can be reduced by limiting aerial application of glyphosate to higher-density buffelgrass stands where susceptible non-target species are no longer present.

INTRODUCTION

Background

Buffelgrass is a warm-season, perennial bunchgrass native to parts of Africa, the Middle East, and Asia (Cook et al. 2005). Because of its rapid response to precipitation (Christie 1975; Ward et al. 2006), drought resistance (Clarke et al. 2005; Nawazish et al. 2006; Sheriff and Ludlow 1984), and greater tolerance of increased grazing pressure over some native grasses (Ludwig et al. 2000), buffelgrass has been nicknamed a “wonder grass” (Hanselka 1988) and has been intentionally introduced in subtropical regions worldwide for forage and erosion control. Buffelgrass has been repeatedly introduced in Australia (Eyre et al. 2009; Friedel et al. 2006; Humphreys 1967; Smyth et al. 2009), the United States (Cox et al. 1988; Cox and Thacker 1992; Daehler and Goergen 2005; Hanselka 1988; Rutman and Dickson 2002; Warren and Aschmann 1993), Mexico (Arriaga et al. 2004; Cox et al. 1988; De la Barrera and Castellanos 2007; Franklin et al. 2006), the Caribbean (Thaxton et al. 2012) and South America (Araujo da Silva Formiga et al. 2012; Blanco et al. 2005; Guevara et al. 2009; Martins et al. 2013; Ruiz and Terenti 2012; Wick et al. 2000).

In many of these regions buffelgrass moves from areas of introduction and becomes an aggressive invader in undisturbed areas (Franks 2002; Olsson et al. 2012a) with many documented unintended consequences. For example, it may aggressively compete with and displace native Sonoran Desert plant species (Eilts and Huxman 2013; Lyons et al. 2013; Morales-Romero et al. 2012), resulting in ecosystem transformation (Humphries et al. 1991; Olsson et al. 2012a) and eventually the potential for altered fire regimes (McDonald and McPherson 2011; McDonald and McPherson 2013; Miller et al. 2010; Schlesinger et al. 2013). Although still a popular reclamation, erosion control and forage grass in some areas

(Bhattarai et al. 2008; Burquez-Montijo et al. 2002; Franklin et al. 2006; Guevara et al. 2009), buffelgrass is gaining attention as an invasive species of concern that threatens biodiversity in subtropical regions worldwide (Marshall et al. 2012). In the Sonoran Desert of Arizona, buffelgrass has become enough of a management priority that multiple spread and management optimization models have been constructed (Bueyuektahtakin et al. 2011; Buyuktahtakin et al. 2014; Frid et al. 2013).

Local buffelgrass populations typically have low genotypic diversity (Gutierrez-Ozuna et al. 2009). They exhibit a high level of phenotypic plasticity, especially in aboveground biomass production in response to water stress (Kharrat-Souissi et al. 2014). This may facilitate invasion and persistence in a range of environments with highly variable precipitation (Arshad et al. 2007; Mnif et al. 2005). The success of buffelgrass as an invader has been attributed to its ease of establishment, rapid growth rate, fast maturation, prolonged flowering periods, prolific seed production, and effective seed dispersal along with long seed dormancy (Franks 2002). Buffelgrass has invaded a variety of sites in the Sonoran Desert, and the efficacy of management approaches may be different at each due to differing environmental conditions, phenologies and plant morphologies that can affect the timing of reproduction, seedling emergence, and plant vegetative growth and senescence. For studies in this dissertation I am particularly interested in the latter as they affect the timing and effectiveness of herbicide uptake and translocation (Bussan and Dyer 1999; Devine et al. 1993; Ross and Lembi 2008).

Predicting greenness

The use of time-series of digital camera images in combination with site specific meteorological measurements to predict phenological status of vegetation in both agriculture and wildland ecosystems is becoming increasingly prevalent (Adamsen et al. 1999; Bater et al. 2011; Crimmins and Crimmins 2008; Ide and Oguma 2010; Ide and Oguma 2013; Kurc and Benton 2010; Laliberte et al. 2007; Richardson et al. 2009; Richardson et al. 2007; Sonnentag et al. 2012; Turner et al. 2006). Time-lapse digital cameras providing a cost effective alternative to daily human observations of plant phenology and allow for monitoring of phenological transitions that can be difficult to forecast without such data. Typical of C_4 perennial grasses in arid environments (Archibald and Scholes 2007; Higgins et al. 2011), buffelgrass growth is largely driven by warm-season rainfall pulses, which are rare and unpredictable in southern Arizona (Phillips and Comus 1999). Buffelgrass remains dormant during most of the year (Olsson et al. 2011), with active growth usually occurring for a two to six week period between July and September (T.M. Bean, personal observation). During this time frame, buffelgrass plants will break dormancy and resume active vegetative growth. This is when buffelgrass plants are most susceptible to chemical control with foliar uptake herbicides like glyphosate, making it an important window of opportunity for regional buffelgrass management. Managers typically rely on *in situ* visual estimates of buffelgrass greenness to schedule herbicide application on a day-to-day basis, with little or no predictive estimation of future susceptibility. The ability to make such predictions of future susceptibility would allow managers more flexibility in prioritizing use of limited resources. To address this management need, I explored the use of time-lapse digital cameras along with site-specific weather data as a cost-effective approach to monitoring the occurrence of greenup and therefore herbicide susceptibility.

Chemical control

Historically, weed management experiments with buffelgrass focused primarily on *avoiding* herbicide injury to buffelgrass being grown as a forage crop (Baur et al. 1977; Bovey et al. 1979; Bovey et al. 1980; Bovey et al. 1984; Bovey and Hussey 1991; Mayeux and Hamilton 1983; Rasmussen et al. 1986). In recent years, studies have begun to evaluate herbicide effects and effectiveness for use in managing buffelgrass invasions (studies focused on maximizing damage to buffelgrass). Australian land managers have examined the efficacy of several herbicides for controlling buffelgrass, including glyphosate, fluazifop and haloxyfop in Queensland (Dixon et al. 2003) and flupropanate in South Australia (Biosecurity 2012). Otherwise these studies have been largely based in Arizona and Texas, perhaps because these two states have advanced invasions in high-value federal lands such as Saguaro and Big Bend National Parks and several other parks, reserves and forests. Of the herbicides evaluated, only glyphosate and imazapyr have been used successfully to kill mature plants in a single application (D. Backer, personal communication, “Herbicide efficacy trials on buffelgrass,” 2009). In Arizona the managers typically report using hand-pump backpack sprayers and mix glyphosate and water at a 2-5% solution (D. Backer, National Park Service; S. Biedenbender, US Forest Service; D. Tersey, Bureau of Land Management; D. Siegel, Pima County; personal communication). Unfortunately, no calibration is used to standardize the amount of solution applied on a per area basis so the actual amount of herbicide applied per area is unknown and probably highly variable (Alam and Hussain 2010; Spencer and Dent 1991). This is true for both on-the-ground herbicide application for buffelgrass control and in the previously mentioned herbicide evaluation studies on buffelgrass (D. Backer, personal communication, “Herbicide efficacy trials on buffelgrass,” 2009).

The advantage of this technique for herbicide application is that it is simple and straightforward, requiring very little special training or calculations to mix and apply the herbicide (Millstein 1995). The disadvantage is that tank pressure and spraying times per plant can vary widely (especially when terrain and buffelgrass densities are not uniform, which they rarely are in wildland settings), resulting in a high range of herbicide being applied on a per area basis with potentially large differences in plant responses (Alam and Hussain 2010), and perhaps most importantly, no clear means for reproducibility of the treatments. To correct for these disadvantages, the alternative is to calibrate backpack sprayer applications for constant pressure, constant nozzle height, known spray swath areas or widths, carrier rates, and speeds of application (Alam and Hussain 2010; Dent and Spencer 1993). Such applications require more training, more calculations, and special equipment, which discourage managers from utilizing calibrated broadcast techniques. For foliar uptake herbicides like glyphosate with no soil activity (Baylis 2000) and little risk from over application, the disadvantages of broadcast outweigh the benefits in wildland settings. However, this has prevented any quantitative estimation of the actual amount of foliar uptake herbicide required to kill buffelgrass, so rates reported to be effective by managers are not comparable and offer little guidance for adjusting tank mixtures to suit different field conditions. Individual plant treatment, unless modified for calibration to regulate herbicide applied per area, may also be a risky technique if soil-mobile herbicides such as imazapyr prove to be effective for buffelgrass control.

Backer and Foster (2007) found that glyphosate was effective at killing buffelgrass plants in Arizona a single application, and it has been the herbicide of choice among managers since active chemical control efforts began in Arizona, likely due to its effectiveness and low potential for non-target damage. However, reliance on glyphosate as the sole herbicide for chemical control of buffelgrass poses several potential problems for managers. First, glyphosate has no soil activity or effect on buffelgrass seeds in the soil that may remain viable for several years (Winkworth 1963; Winkworth 1971) and infested areas need to be retreated for long-term control. Second, glyphosate is non-selective and may damage some non-target species, depending on the amount of chemical exposure and treatment frequency. Third, glyphosate is symplastically translocated, requiring active vegetative growth for optimum action on meristematic tissue (Ross and Lembi 2008). Potential limitations of glyphosate herbicide justify the exploration of alternative herbicides for chemical control of buffelgrass, and to ensure that the results of these studies can be utilized across a maximum number of settings, it is essential that they not be performed with the traditional IPT application methods.

Helicopter broadcast herbicide application

Buffelgrass infestations often occur in dense stands on steep rocky slopes that can be practically inaccessible from the ground or pose a safety hazard to field crews. As of 2009, over 4,000 ha of buffelgrass existed in southern Arizona on federal land alone and at most, 100's of ha (< 10% of the total) are being treated on an annual basis with pulling or chemical IPT methods (D. Backer, USDI National Park Service; S. Biedenbender, USDA Forest Service; D. Tersey, USDI Bureau of Land Management; personal communications). In other words, although ground-based herbicide application is capable of treating much more area per unit time than manual control, neither can keep pace with buffelgrass spread.

A potential solution to constraints on managers' ability to treat enough area in a given year to keep pace with spread rates is the use of helicopter broadcast aerial herbicide application. Aerial herbicide application is routinely used for weed control in agriculture and forestry, with such applications being most successful when weed infestations are homogenous and large in area with uniform terrain (Payne 1993; Reddy et al. 2010; Riley et al. 1991; Yates et al. 1978; Zutter et al. 1988). However, buffelgrass infestations in the Sonoran Desert offer several unique challenges, including steep, rugged terrain, relatively small target areas, extremely hot, low-humidity conditions, and the protection of native plants unique to this landscape, particularly saguaro, which can reach over 15 m in height and thus also pose a safety threat to the helicopter operator and require that herbicide must be applied from an unusually high altitude (Thistle et al. 2014). Three federal agencies, two local jurisdictions and University of Arizona scientists collaborated to design and implement a field trial to evaluate helicopter broadcast herbicide application for buffelgrass control. (Backer et al. 2012). Because this was a broadcast application, effects on native non-target vegetation were also a concern. We utilized this helicopter broadcast herbicide application trial to evaluate the feasibility of aerial herbicide application for buffelgrass control and potential environmental impacts to assist land managers in completing legal documentation required for an Environmental Assessment or Environmental Impact Statement.

Summary

The impacts of buffelgrass invasion are well documented and considerable resources are being spent on control efforts in the Sonoran Desert of Arizona by both federal agencies and local jurisdictions. Unfortunately, current efforts are not sufficient to keep pace with rates of spread, and buffelgrass populations continue to expand rapidly.

Though effective at small scales, current techniques for management need to be updated to make progress in the region. Research into improved management is clearly called for. If applied, the results contained in this dissertation have the potential to dramatically improve buffelgrass management. First, we provide predictive capabilities for the timing of active buffelgrass growth and herbicide susceptibility. This will allow managers more time to prioritize the allocation of limited resources in the form of herbicide spraying crews. Second, we provide a precise and accurate glyphosate rate that will kill mature buffelgrass plants in a single application and can be reproduced by managers with simple calibration steps. We also screen alternative herbicides to glyphosate and find a potential herbicide that is not dependent on active growth of buffelgrass and can therefore be applied yearlong, greatly increasing the amount of area that can be controlled on an annual basis. Third, we evaluate a helicopter broadcast herbicide application trial, which is a method of herbicide application that can also dramatically increase area controlled on an annual basis, as well as allow access to previously inaccessible infestations.

As an example, a manager may have several locations containing buffelgrass infestations in their jurisdiction. Using the predictive green-up modeling from the first study in this dissertation, the manager can allocate spraying crews to specific locations that are susceptible to herbicide application without spending resources to visit those sites first, saving time and funds as many sites are likely to be remote and difficult to access. The manager can also use the predictive green-up models to schedule costly helicopter applications with surety that buffelgrass will be susceptible to the application. Using herbicides and rates from the second study, the manager can be assured that mature plants will be killed at that location and a second visit during the season will be unnecessary unless it is for the purpose of targeting seedlings. Further, some herbicides (imazapic and imazapyr) from the second study can provide preemergence control of buffelgrass, eliminating the need to revisit the site for control of seedlings in the current season or for 12 months following herbicide application. The efficacy of dormant season application of imazapyr has also been demonstrated to be effective in killing mature buffelgrass plants and allows managers to utilize the entire calendar year for buffelgrass control, greatly expanding the potential to stop or slow buffelgrass population expansion at a regional level. Because imazapyr is nonselective, has a long soil residual and is highly soil mobile, this herbicide is most appropriate for controlling older, denser buffelgrass infestations where the potential for native non-target vegetation damage is minimal. Finally, the third study demonstrates that buffelgrass can be controlled and likely killed with helicopter application of glyphosate and details the effects of different glyphosate deposition rates on certain species of native non-target vegetation. Helicopter application offers a viable option to managers to treat large areas of buffelgrass in a single season and to reach buffelgrass populations that would otherwise be inaccessible. However, glyphosate application rates varied dramatically from deposition rates measured on the ground. The study compares buffelgrass response to glyphosate deposition rates from helicopter application to rates applied from the ground and concludes that buffelgrass can be killed if deposition can be increased. This study discusses ways to optimize helicopter application of glyphosate without increasing application rates, namely by applying only in the early morning hours before the atmosphere destabilizes, and also offers suggestions to improve the monitoring of the effects of such applications on both buffelgrass and native non-target vegetation.

EXPLANATION OF THE DISSERTATION FORMAT

The goal of this dissertation is to understand (i) the factors influencing the timing of buffelgrass greenup and senescence and thereby predict susceptibility to foliar uptake herbicides like glyphosate; (ii) the efficacy of alternative herbicides for improved chemical control; and (iii) the effectiveness of helicopter broadcast herbicide application for controlling buffelgrass and its effects on native non-target vegetation. The results of this dissertation are presented as three separate, appended manuscripts. Appendix A addresses the relationship between antecedent weather and the timing of buffelgrass greenup and senescence using automated time-lapse photography in order to predict susceptibility to foliar uptake herbicides. Appendix B considers the effectiveness of improved approaches to chemical control of buffelgrass, including herbicides and herbicide mixtures that may provide preemergence control, increased selectivity and decreased native non-target damage, or may be applied during dormancy. Appendix C assesses the efficacy of aerial herbicide application for buffelgrass control and the potential for damage to native non-target vegetation.

PRESENT STUDY

The specific research questions asked, methodologies used, results obtained, and conclusions drawn from this study are presented in the manuscripts appended to this dissertation. The following is a brief summary of each manuscript. Although the appendices have co-authors, the dissertation as a whole represents my original, independent work. I designed the research, collected data, performed analyses, and wrote the manuscripts. The contribution of each co-author is described below.

Appendix A: *Predicting buffelgrass greenup and senescence using antecedent weather and ground-based remote sensing* is a manuscript to be submitted to *Invasive Plant Science and Management*. The predominant herbicide used to control buffelgrass is glyphosate, which requires active vegetative growth when applied for optimum uptake and translocation to meristematic tissue. However, control efforts are complicated by managers' inability to accurately predict the occurrence and duration of active buffelgrass growth periods. Our objective was to develop tools to predict the timing and length of future active growth during the summer based on day of year and antecedent weather for a given location and season. Use of these predictive tools will allow for herbicide application efforts to be directed to time periods and sites where plants are most susceptible. We evaluated relationships among temperature, precipitation, relative humidity, and plant greenness (a proxy for active plant growth, visually estimated as the ratio of green to dead or dormant tissue). This was done using daily time-lapse digital photography in stands of buffelgrass at three sites in the Sonoran Desert near Tucson, AZ that were representative of habitats currently infested by buffelgrass in the region. These included a level (water-accumulating) basin floor site with a deep soil and that received supplemental moisture as precipitation runoff from adjacent areas, a slope site receiving relatively higher seasonal precipitation, and a more xeric slope site. We collected data over the 2012 and 2013 summer growing seasons (June-October), using the 2012 data to train predictive multiple regression models and 2013 data to test model prediction accuracy. Model effectiveness was gauged by prediction agreement with observations at a threshold of 60% greenness, which represents the phenological stage where buffelgrass becomes susceptible to mortality with glyphosate application. Accuracy of models constructed using data from all sites included differed only minimally from models that were site-specific. Each site had different patterns of model accuracy, as measured by the probability that the predicted and observed greenness would both be either above or below 60% for a given date. We were able to correctly predict greenness above or below the 60% threshold at 81 to 95% for the basin floor site up to 28 days into the future, at 61-88% for the slope site receiving higher precipitation up to 14 days into the future, and at 62-82% for the more xeric slope site at 0-28 days into the future.

Co-author Steven Smith, dissertation director, assisted with model development and read and commented on manuscript drafts. Co-author Perry Grissom provided comments and edits on an earlier draft of this manuscript. This research was funded by a National Park Service Fuels Reserve Fund Research Request.

Appendix B: *Buffelgrass control with glyphosate, clethodim, imazapic and imazapyr* is a manuscript to be submitted to *Invasive Plant Science and Management*. Chemical control of buffelgrass in Arizona's Sonoran Desert primarily currently relies on glyphosate, which has no preemergence activity, is not selective for grasses, and cannot be applied when buffelgrass is dormant. To address these issues, we conducted a series of replicated field experiments from 2010 to 2013 at an undisturbed wildland site and a former agricultural field near Tucson, AZ to investigate herbicides and herbicide mixtures, application rates, and application timing effects for control of buffelgrass. We evaluated the effects of different rates of two herbicides (imazapic and clethodim), alone or in combination with different rates of glyphosate, for pre- (imazapic only) and postemergence control of buffelgrass. We also evaluated growing- and dormant-season (summer and winter, respectively) application of imazapyr for pre- and postemergence control of buffelgrass. We used CO_2 pressurized sprayers to apply herbicides at known pressures, speeds, and carrier rates to ensure accurate broadcast application rates. Our results indicate that when applied alone, glyphosate rates of 2.52 kg acid equivalent (ae) ha^{-1} (2,25 lb ae ac^{-1}) are needed to kill mature plants in a single application. Lower rates of glyphosate were effective when combined with imazapic. Imazapic did not kill mature buffelgrass plants even at the highest label rate, although this rate (0.21 kg ae ha^{-1} [0.19 lb ae ac^{-1}]) did suppress shoot growth at 36 months after treatment (MAT). Clethodim did not have any effect on buffelgrass, even at the highest label rate. At one of two sites, imazapyr killed mature buffelgrass plants 6 MAT when applied during the dormant season at 0.56 kg ae ha^{-1} (0.50 lb ae ac^{-1}), or 12 MAT when applied during the growing season at 1.12 kg ae ha^{-1} (1.00 lb ae ac^{-1}). Imazapyr provided effective preemergence control of buffelgrass at all rates 6 and 12 MAT. Thus, while glyphosate can kill mature buffelgrass plants after a single application, its utility is limited by the short and unpredictable growing season when buffelgrass is susceptible and by its lack of soil activity. Imazapyr shows promise for year-round pre- and postemergence control of buffelgrass.

Co-author William McCloskey provided herbicides and application equipment and assisted with plot installation, treatment applications, and monitoring. He and co-author Steven Smith, dissertation director, provided experimental design advice, reviewed data analyses, and commented on manuscript drafts. This study was funded through the USDA Forest Service Pesticide Impact Assessment Program (projects R3-0110 and R3-2012-02).

Appendix C: *Evaluation of buffelgrass and native vegetation response to helicopter-applied glyphosate in the Sonoran Desert* is a manuscript to be submitted to *Invasive Plant Science and Management*. Rapid spread of buffelgrass in the Sonoran Desert is outpacing ground-based management efforts in many situations. Further, many buffelgrass populations are located on steep slopes in backcountry areas that are essentially inaccessible on foot. To overcome these limitations, federal agencies and two local jurisdictions conducted a field trial that applied glyphosate by air in the southern Tucson Mountains southwest of Tucson, AZ in the summer of 2010. We subsequently investigated the efficacy of the helicopter broadcast applied glyphosate for buffelgrass control and the concomitant injury these applications caused to native non-target vegetation. Two rates of glyphosate, 1.13 and 2.23 kg ae ha^{-1} (1.01 and 1.99 lb ae ac^{-1}), were each sprayed in carrier volumes of 46.9 and 93.8 L ha^{-1} (5 and 10 gal ac^{-1}) of water for a total of four treatments. However, a related study (Thistle et al., 2014) found that the rates of glyphosate deposited on the ground were much less than what was released from the helicopter (26-76% of released) due to the height of spray release, droplet size and environmental factors. Sites were chosen to contain both buffelgrass, which was monitored for plant health one-year following glyphosate application, and representative non-target vegetation; over 1,600 individual non-target plants were marked and monitored for at least two years after the glyphosate applications. Buffelgrass was also monitored under paloverdes above 1.5 m in height and in plant interspaces to evaluate canopy effects. Glyphosate deposition decreased with increasing time of day, which was likely due to atmostpheric instability (Thistle et al. 2014). These times also coincided with all 46.9 L ha^{-1} carrier rate treatments, potentially confounding carrier rate as a model factor. Buffelgrass control increased with increasing glyphosate deposition rate and glyphosate deposition rate had a significant interaction with plant location in relation to paloverde canopies. Saguaros and other cacti were not affected by any of the treatments in year one or two post glyphosate application. Generally, response of non-target vegetation followed a trend where shrubs of increasing height incurred less damage (measured as change in plant greenness) from herbicide treatments than did smaller life forms. Brittlebush, limberbush, ocotillo, little leaf paloverde, triangle leaf bursage, and wolfberry were the only non-target plants in the study to experience damage from any of the treatments. Buffelgrass had a higher rate of increasing damage per kg ae ha^{-1} glyphosate deposition than non-target vegetation except limberbush. Buffelgrass injury was consistent with ground-based applications when deposited rate of glyphosate is considered. Although damaged, buffelgrass was not killed at rates applied in this study. The protocols used in this study need to be modified to kill buffelgrass plants in a single application. Although raising glyphosate rates may be required, as a first step helicopter applications should be made early in the morning before thermal updrafts on steep slopes disperse spray droplets. Larger spray droplets and anti-evaporant adjuvants may also help more glyphosate reach the ground. This may result in greater injury or possible mortality of certain non-target species if present in buffelgrass stands. Finally, non-target damage can be reduced by limiting aerial application of glyphosate to higher-density buffelgrass stands where susceptible non-target species are no longer present.

Co-author Steven Smith, dissertation director, provided experimental design advice, reviewed data analyses, and commented on manuscript drafts. Co-author Harold Thistle funded manuscript development and study implementation. Co-author Dana Backer coordinated study implementation and performed the majority of the data collection.

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APPENDIX A: Predicting buffelgrass greenness with empirical models based on data derived from time-lapse digital photography and long-term weather stations

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Abstract

Buffelgrass is a perennial C_4 bunchgrass that can be invasive in subtropical regions worldwide. Buffelgrass control is a priority in many areas because of its rapid invasion rate, tendency to displace native vegetation, and the associated fire risk to native plant communities, adjacent developed areas and their associated infrastructure. Mechanical control is often impractical and unable to keep pace with the exponential rate of growth often seen in buffelgrass populations. Chemical control currently offers the most promise for successful and cost-effective management on a regional scale. The predominant herbicide used to control buffelgrass is glyphosate, which requires active vegetative growth when applied for optimum uptake and translocation to meristematic tissue. However, control efforts are complicated by managers' inability to accurately predict the occurrence and duration of active buffelgrass growth, which occurs in short bursts following summer precipitation. Our objective was to develop tools to predict the timing and length of future active growth during the summer based on day of year and antecedent weather for a given location and season. Predictive tools will allow herbicides to be applied during time periods and at sites where plants are most susceptible. We evaluated relationships among temperature, precipitation, relative humidity, and plant greenness (a proxy for active plant growth that is visually estimated as the ratio of green to dead or dormant tissue). This was done using daily time-lapse digital photography in stands of buffelgrass at three sites in the Sonoran Desert near Tucson, AZ that were representative of habitats currently infested by buffelgrass in the region. These included a level (water-accumulating) basin floor site with a deep soil that received supplemental moisture as precipitation runoff from adjacent areas, a slope site that received relatively greater seasonal precipitation, and a more xeric slope site. We collected data during the 2012 and 2013 summer growing seasons (June-October), using the 2012 data to train predictive multiple regression models and 2013 data to test model prediction accuracy. Model effectiveness was gauged by prediction agreement with observations at a threshold of 60% greenness, which is a conservative estimate of the phenological stage where buffelgrass becomes susceptible to mortality with glyphosate application (T. Bean, personal observation). Accuracy of models constructed using data from all sites differed minimally from models that were site-specific. Each site had different patterns of model accuracy, as measured by the probability that the predicted and observed greenness would both be either above or below 60% for a given date. We were able to correctly predict greenness above or below the 60% threshold at 81 to 95% for the basin floor site up to 28 days into the future, at 61-88% for the slope site receiving higher precipitation up to 14 days into the future, and at 62-82% for the more xeric slope site at 0-28 days into the future.

Nomenclature: Glyphosate; buffelgrass, *Cenchrus ciliaris* L.

Key words: C_4 perennial bunchgrass, chemical control, digital image, greenness, phenology time series.

Management Implications:

The area occupied by buffelgrass (*Cenchrus ciliaris* L.) populations has expanded exponentially in certain areas of the Sonoran Desert since at least the late 1980s. Manual removal approaches are of limited effectiveness due to high labor requirements given the rugged sites involved and the large area targeted for management. Chemical control currently relies on glyphosate, which is a foliar applied, symplastically mobile herbicide that requires active vegetative growth for optimal uptake and translocation. Unfortunately, such growth occurs in buffelgrass during a brief and unpredictable window of time following the onset of the summer rainy season in southern Arizona. This period of herbicide susceptibility typically occurs within two to six weeks per year, and is often comprised of multiple non-continuous time periods. Our research provides land managers in the Sonoran Desert with the ability to objectively predict herbicide susceptibility of buffelgrass populations during the summer growing season on slopes in the Santa Catalina Mountains up to 7 to 14 days from present, and at Tucson Mountain and Tucson Basin floor sites up to 28 days from present. This is done using simple main effects-only multiple regression models that rely on various combinations of day of year and some measure of antecedent humidity, precipitation, and temperature. While temperature and humidity are relatively uniform in the Tucson region during the summer months, precipitation varies widely in space and time. Managers are encouraged to establish rain gauges near buffelgrass populations targeted for future management to facilitate prediction of herbicide susceptibility using these tools.

Introduction

Buffelgrass (*Cenchrus ciliaris* L.) is a perennial African bunchgrass that is rapidly spreading throughout parts of the Sonoran Desert as a result of both deliberate planting (Franklin et al. 2006; Franklin and Molina-Freaner 2010) and invasion into undisturbed areas (Olsson et al. 2012b). Once established, it often aggressively competes with and may displace native Sonoran Desert plant species (Eilts and Huxman 2013; Lyons et al. 2013; Morales-Romero et al. 2012), resulting in ecosystem transformation (Olsson et al. 2012a) and eventually the potential for altered fire regimes (McDonald and McPherson 2011; McDonald and McPherson 2013; Schlesinger et al. 2013). Although local populations likely have low genotypic diversity (Gutierrez-Ozuna et al. 2009), buffelgrass exhibits a high level of phenotypic plasticity allowing it to invade and persist in a range of environments with highly variable precipitation (Arshad et al. 2007; Kharrat-Souissi et al. 2014; Mnif et al. 2005). As such, buffelgrass has invaded a variety of sites in the Sonoran Desert, and the efficacy of management approaches may be different at each due to differing environmental conditions, phenologies and plant morphologies that can affect herbicide uptake and translocation (Bussan and Dyer 1999; Devine et al. 1993; Ross and Lembi 2008). This is relevant because current management approaches consist solely of chemical and manual control, and manual control is of limited efficacy due to its high cost, the rugged nature of occupied environments, and the rapid expansion of buffelgrass populations.

Typical of C_4 perennial grasses in arid environments (Archibald and Scholes 2007; Higgins et al. 2011), buffelgrass growth is largely driven by warm-season rainfall pulses, which are rare and unpredictable in southern Arizona. Thus, buffelgrass remains dormant during most of the year (Olsson et al. 2011), with active growth usually occurring for a two to six week period between July and September (T.M. Bean, personal observation; Figures 1 and 2). Though timing of these events are difficult to predict, they are sufficient to facilitate germination and seedling establishment in most years resulting in constant rates of spread (Olsson et al. 2012b). The majority of invasive plant managers in the Sonoran Desert region of Arizona target buffelgrass plants and populations that have reached at least 50-60% green tissue so that vegetative growth is substantial enough to ensure sufficient foliar uptake and translocation of glyphosate to kill buffelgrass (T. Bean, personal observation). Managers typically rely on *in situ* visual estimates of buffelgrass greenness to schedule herbicide application on a day-to-day basis, with little or no predictive estimation of future susceptibility. The ability to make such predictions would allow managers more flexibility in optimizing use of limited resources.

The objective of this research was to develop a tool that managers can use to predict when and where buffelgrass populations would experience active vegetative growth and be susceptible to chemical control with glyphosate. Digital time-lapse cameras have been successfully used as a cost-effective (Harer et al. 2013; Ide and Oguma 2013) and automated (Migliavacca et al. 2011; Zerger et al. 2012) approach to monitoring phenological changes in a variety of species and ecosystems. In this research we used digital time-lapse cameras to obtain image data that allowed us to model the complexity of buffelgrass growth, which is a major obstacle for effective chemical control. We sought to answer three questions. First, can time-lapse digital photography be used to track daily changes in buffelgrass greenness (as a proxy for active plant growth) and senescence? Second, what variables are the best predictors of buffelgrass greenness? Finally, can these relationships be used to predict the timing and length of periods of future greenness and therefore periods of glyphosate herbicide susceptibility in buffelgrass populations at specific sites in the Sonoran Desert of southern Arizona? To answer these questions we monitored daily weather and gathered daily image data from digital camera stations for three buffelgrass populations representing a range of typical infested habitats in the region. With this data, we created a time-series dataset and used multiple regression models trained with first-year data to predict greenness in the second year.

Materials and Methods

Locations. We selected three sites in the Arizona Upland subdivision of the Sonoran Desert near Tucson, AZ for this research. These sites were selected to be representative of typical habitats infested by buffelgrass in the region and because of their proximity to long-term weather stations. The Sabino Canyon site is located at 32.317641, -110.810477 on the Coronado National Forest in the Santa Catalina Mountains, northeast of metropolitan Tucson at 860 m (2830 ft) elevation (Figure 3). This site is located on a very steep (50 to 60%), excessively drained, southwest-facing rocky slope with shallow (15 cm or 6 in), granitic, very gravelly sandy loam soil, and was the most mesic site, receiving 343 and 276 mm (13.5 and 10.9 in) of annual precipitation and 241 and 144 mm (9.5 and 5.7 in) of summer precipitation in 2012 and 2013, respectively. (Typically, the summer growing season in the Sonoran Desert region of southern Arizona is initiated in June or July and ends in September or October. Summer precipitation in this research included all precipitation in these five months.) The native plant community is dominated by littleleaf paloverde (*Parkinsonia microphylla* Torr.) and saguaro (*Carnegiea gigantea* [Engelm.]

Britton & Rose). The Sabino Canyon site is similar in elevation, slope, soil and seasonal precipitation totals to many buffelgrass-infested areas in the Santa Catalina and Rincon Mountains (T.M. Bean, personal observation). The second site (32.131853, -110.958371) is located near the Tucson International Airport (hereafter "Tucson Airport") at 770 m (2540 ft) elevation on a nearly level relict fan terrace with slightly deeper (20 cm or 8 in) gravelly fine sandy loam soil (Figure 3). This site received 201 and 215 mm (7.9 and 8.5 in) of annual precipitation and 153 and 95 mm (6.0 and 3.7 in) of summer precipitation in 2012 and 2013, respectively. This site also receives supplemental moisture as precipitation runoff from adjacent lands, much of which are paved surfaces. This site is dominated by creosote bush (*Larrea tridentata* [Sessé & Moc. ex DC.] Coville) and velvet mesquite (*Prosopis velutina* Wooton). The site is similar in slope, soils, and seasonal precipitation amount to other buffelgrass-infested areas on the Tucson Basin floor that are also often embedded within urban and suburban developments (T.M. Bean, personal observation). The third site is located in the southeastern edge of the Tucson Mountains on a steep (40 to 50%), well-drained, east-facing slope at 32.217555, -111.002810 on Tumamoc Hill at 800 m (2630 ft) elevation on a very shallow (10 cm or 4 in), basaltic, extremely cobbly loam soil (Figure 3). Tumamoc Hill received 213 and 235 mm (8.4 and 9.3 in) of annual precipitation and 154 and 112 mm (6.1 and 4.4 in) of summer precipitation in 2012 and 2013, respectively. As with Sabino Canyon, Tumamoc Hill is dominated by littleleaf paloverde and saguaro. However, similar to other Tucson Mountain sites, this site is lower in elevation and generally receives less summer rainfall than sites in the Catalina Mountains.

Data collection and processing. Study sites were established in June 2012 and data were collected through November 2013. Each site was located within 500 m (1600 ft) of a long-term (>20 yr of data) weather station that collected daily precipitation, temperature, and relative humidity data. These data were used to calculate daily mean, maximum and minimum values, as well as cumulative means from 5 to 30-day mean, maximum and minimum daily values. We arbitrarily divided this data into 5-day intervals (e.g. 5-, 10-, 15-, 20-, 25-, and 30-day mean maximum temperature). Three time-lapse cameras (Moultrie I-60, Moultrie ® Feeders, Alabaster, AL) were installed at each study site. Our camera placement and monitoring approach was similar to that used by Kurc and Benton (2010). All cameras were pointed north and spaced to capture non-overlapping images of the same buffelgrass patch that was dominated by mature (> two-year-old) plants. Cameras were placed within 5 m (16 ft) of buffelgrass plants at a height of approximately 0.6 to 0.9 m (2 to 3 ft). Each buffelgrass patch was several hectares in size with individual buffelgrass plants having a density of 3 to 5 plants m^{-2} (0.5 plants ft^{-2}). Cameras were set to take one photograph per hour. We used a MATLAB (MATLAB 2014a, MathWorks, Inc., Natick, MA) script (available upon request from the corresponding author) to batch process the images by first selecting only images taken between the hours of 11:00 and 13:00 h, to minimize differences in sun angle and shading. This script then designated a polygon (polygons varied in size for each camera view according to the relative proportion of buffelgrass present) in each camera view from which to extract red, green, and blue (RGB) pixel digital numbers (DN), masking the remainder of the image (Sonnentag et al. 2012). Examples of camera images and polygons can be seen in Figures 4a, b, and c. The result was a daily mean value for each RGB pixel channel for each camera and day. These daily mean values were averaged across cameras for each site to provide a single value for each RGB pixel DN by day at each site (Richardson et al. 2009) (Figure 5a, b, and c). From these daily means of

RGB pixel DN at each site, we calculated quantitative daily approximations of "greenness" using the "excess green" (EG) and "green chromatic coordinates" (GCC) indices (Gillespie et al. 1987; Richardson et al. 2007; Woebbecke et al. 1995) (Figure 5a, b, and c; Equations 1 and 2):

$$EG = 2 * Green\,DN - (Red\,DN + Blue\,DN) \quad [1]$$

$$GCC = Green \frac{DN}{Red\,DN + Green\,DN + Blue\,DN} \quad [2]$$

Both greenness indices followed the same trends and were virtually indistinguishable, but EG had a slightly higher correlation with visual estimates of greenness taken during monitoring (data not shown). To translate from EG index to greenness (%), we developed a training set of images representing six approximate classes of visual greenness: 0, 1 to 20, 21 to 40, 41 to 60, 61 to 80, and 81 to 100% green (Figure 6). These classes are based on the maximum amount of separation in greenness we have found that researchers can consistently identify visually in the field with regular practice. Finer-scale greenness classes usually result in a lack of consensus among researchers (Kennedy and Addison 1987; Macfarlane and Ogden 2012; Sykes et al. 1983). We asked a group of four researchers with ample experience scoring buffelgrass greenness in the field to standardize their evaluation using the training set. We then created a random selection of camera images, stratified by site and camera (90 images total or 10 images for each of three cameras at each of three sites). Next, the images were independently scored for visual greenness (using the six classes mentioned above) by the researchers. Once greenness classes were identified, the midpoint of the class was used to approximate a continuous variable in analysis (e.g., a plant in the 1 to 20% green class would be treated as 10% green). These midpoints were averaged and regressed against the EG values for the images using a quadratic equation, creating a daily visual greenness estimate for the remaining dataset (Figure 7).

Model selection, training, and validation. Buffelgrass is phenotypically plastic (Arshad et al. 2007; Kharrat-Souissi et al. 2014; Mnif et al. 2005); buffelgrass seedlings have been observed to emerge and mature plants have been observed to green-up (transition from dormancy to active vegetative growth, starting with emergence of new leaves), flower and senesce at almost any time of year in the Sonoran Desert of Arizona. The only two seasonal phenological states that can be reliably predicted in this region are a period of dormancy sometime in May through June, and a period of active growth sometime in July through September. To maximize utility for managers and minimize the influence of possible rare drivers of non-summer green-up, we focused on predicting greenness during the summer growing season. We defined the start of this season as the first of three consecutive days when the average dew point temperature (at the weather station nearest the study site) was greater than 12°C (54°F). This was the historic definition of the start of the monsoon used by NOAA in Arizona until 2008 (NOAA 2014), and we found that greenup occurred within one week of this date for each of the three sites in both 2012 and 2013. For Sabino Canyon, the Tucson Airport, and Tumamoc Hill these dates were July 5, July 4 and June 26 in 2012, respectively and July 6, July 7, and July 2 in 2013, respectively. We defined the end of this season as October 15, which was the date by which all sites had returned to approximately pre-summer growing season greenness levels in 2012 and 2013. For model training and refinement, we also restricted our dataset to 2012 values only, reserving the 2013 data for model validation in order to avoid autocorrelation effects within time series data and model overfitting.

Similar to Smith et al. (2013) and Sanche and Lonergan (2006), we used the PROC VARCLUS procedure in SAS (SAS 2012, Version 9.3; SAS Institute, Inc., Cary, NC) to objectively identify redundancies among independent variables (day of year and weather variables) with hierarchical clustering on oblique centroid components. This was performed for all sites together and for each of the three study sites individually, resulting in a four subsets of variables from which to construct predictive multiple linear regression models. For each subset, we developed individual models to predict greenness at 0, 7, 14, 21 and 28 days in the future (Table 2 to 5). We chose weekly intervals for modeling because we wanted to minimize model redundancy by having intervals that were too narrow, but would still remain relevant for land managers planning herbicide application efforts. Each model contained only one variable at most for temperature, relative humidity and precipitation to avoid inclusion of multiple highly correlated variables. All independent variables were untransformed and unstandardized. Only main effects models (no interactions among effects) were included to reduce the probability of model over-fitting to a specific site or year. For the models that included all sites, an additional binomial nominal variable was created to differentiate slope vs. basin sites. Assuming that managers would not necessarily have been to the field to assess greenness in areas targeted for glyphosate herbicide application, past and present greenness were not included in the models. Once variable reduction was accomplished, we used the Standard Fit Least Squares option in the Fit Model platform in JMP (JMP Pro, Version 9.0, SAS Institute Inc., Cary, NC) to iteratively evaluate models with different combinations of effects from the reduced subset of variables. We removed non-significant effects or effects that were correlated (>0.7) with other, more significant effects using the Correlations option on the Multivariate platform in

JMP. When multiple effects representing the same weather phenomena (precipitation, temperature, relative humidity, etc.) were both significant and not correlated with each other, we selected the best combination of effects by iteratively minimizing the AIC_c (small-sample-size corrected version of Akaike information criterion) statistic during model selection (Akaike 1974). The Akaike information criterion is a measure of the relative quality of a statistical model for a given set of data that deals with the trade-off between the goodness of fit of the model and the complexity of the model. As an example, equations for the models that predict greenness at Sabino Canyon (Table 3; Equation 3), Tucson Airport (Table 4; Equation 4), and Tumamoc Hill (Table 5; Equation 5) at day zero (present day) are:

$$Sabino\ Canyon\ greenness\ at\ day\ zero = -169.27 + 0.37(Day\ of\ \ year) + 0.42(Mean\ 20\ day\ precipitiation) + 3.00(Mean\ 15\ day\ minimum\ temperature) + 1.25(Mean\ 25\ day\ mean\ relative\ humidity)\ [3]$$

$$Tucson\ Airport\ greenness\ at\ day\ zero = -308.95 = 0.56(Day\ of\ \ year) + 0.36(Mean\ 25\ day\ precipitation + 5.42(Mean\ 15\ day\ mean\ temperature) + 1.72(Mean\ 25\ day\ mean\ relative\ humidity)\ [4]$$

$$Tumamoc\ Hill\ greenness\ at\ day\ zero = -4.79 + 0.52(Mean\ 20\ day\ precipitation) + 1.05(Mean\ 20\ day\ mean\ relative\ humidity)\ [5]$$

As previously stated, the majority of invasive plant managers in the Sonoran Desert region of Arizona target buffelgrass plants and populations that have reached at least 50-60% green tissue so that vegetative growth is substantial enough to ensure sufficient foliar uptake and translocation of glyphosate to kill buffelgrass. Models were tested with 2013 data and their accuracy was evaluated based the probability that both model-predicted and observed values were simultaneously above or below the 60% greenness threshold representing herbicide susceptibility on a given day in 2013 (Figure 8a, b, and c; Table 1). This was accomplished using a conditional formatting ("if/then") script written in JMP that produced a binomial response of "1" each time the predicted and observed values were simultaneously above or below the 60% greenness threshold, otherwise returning a value of "0." The frequency of the "1" response represented the probability that the predicted model was "correct," meaning that the model predictions and actual observations were in agreement as to whether the buffelgrass population would be susceptible to glyphosate application. Model significance was evaluated using a nonparametric one-sample two-tailed binomial test where agreement would occur with probability 0.5 if the model had predictive value no greater than a random choice (VassarStats: Website for Statistical Computation, Binomial Probability calculator with two-tail exact test, http://vassarstats.net/binomialX.html).

Results and Discussion

Approximation of visual greenness. A quadratic regression of the visual greenness in the training set images by EG (Excess Green Index) provided acceptable explanation of the variation in visual greenness with an R^2 of 87% (Figure 7). Based on the steep slope of the regression at lower values of EG, lower values of visual greenness (<60%) were more difficult to differentiate than higher values using digital image-derived EG. However, this may be irrelevant given the management significance of the 60% visual greenness threshold mentioned above. For management purposes, the exact greenness is not important, only if the buffelgrass population is above 60% green and therefore susceptible to glyphosate herbicide, or below 60% green, and therefore less susceptible. This assumes that greenness is a proxy for active vegetative growth, which is required for optimal translocation of symplastically translocated herbicides like glyphosate (Devine et al. 1993; Ross and Lembi 2008). The regression provides good differentiation of higher visual greenness values, but more importantly it successfully differentiates visual greenness values above and below this threshold (93% accuracy, P<0.0001, One-sample binomial test), which represents the cutoff where managers are likely to make decisions on whether to mobilize spray crews. However, this regression may be of limited use in detecting minor fluctuations in visual greenness. An example of this limitation may be associated with estimating responses to small amounts of precipitation or in detecting small visual greenness changes during the springtime when temperatures may not be optimal for growth of a C_4 grass but soil moisture is otherwise sufficient for growth.

Model selection. For all models, variable reduction resulted in four clusters of related variables including a day of year variable and one or two variables each for temperature, relative humidity, and precipitation (Tables 2, 3, 4, and 5). Using variable clustering in PROC VARCLUS and iterative modeling to eliminate correlated and non-significant variables, and to minimize AIC_c statistics, the all sites combined, Sabino Canyon, Tucson Airport, and Tumamoc Hill data were reduced to include four to six effects including intercept (Tables 2 to 5). Not surprisingly, models for different sites and forecasting periods performed better (in terms of predicted and observed values having a higher probability of simultaneously being either above or below the 60% greenness threshold) with different model effects. This is likely explained in part by site differences in soil characteristics, slope and slope aspect, site potential for capturing precipitation runoff, elevation, microhabitat factors, and plant population factors like age of stand, and plant density. For example, Sabino Canyon received the most summer precipitation (241 mm in 2012 and 144 mm in 2013), but probably was unable to retain much moisture (data not shown) within the soil profile because of the steep slopes and shallow soils at this site. The Tucson Airport received 63 and 65% (2012 and 2013, respectively) *less* summer precipitation than Sabino Canyon, but received additional moisture as runoff from adjacent surfaces and has deeper soils, likely resulting in higher soil moisture in the profile for longer periods of time. The Tucson Airport and Tumamoc Hill received similar summer precipitation but like Sabino Canyon, Tumamoc Hill has steep slopes and shallow soil and was likely unable to retain much moisture within the soil profile.

Model prediction. Performance of model predictions was measured as the probability of the predicted (2012 models) and observed (2013 data) values being either both above the 60% greenness threshold or both below the 60% greenness threshold (i.e., the probability that the predicted and observed values were in "agreement" at the level of being both above or both below 60% greenness) for any single date of prediction. The all sites combined models generally predicted greenness observed in 2013 with similar accuracy as the individual site models (Table 1). The only two exceptions are the 14-day future models for Sabino Canyon and Tumamoc Hill. In the case of Sabino Canyon, there was a 61% probability that the site-specific model predictions and the observed greenness from 2013 were simultaneously above or below the 60% greenness threshold. However, the probability that the all sites combined model predictions were in similar agreement with the 2013 observations was nonsignificant (Table 1). Conversely, for Tumamoc Hill, the site-specific model was nonsignificant at 14 days, while the all sites combined model produced a significant prediction of 62%. Excluding these two cases, there are no obvious benefits or drawbacks to using the all sites combined or site-specific models for these data. We recommend that managers use the model most similar to their target buffelgrass population's habitat (or the all sites combined model if their site is intermediate).

Predictions of future greenness were most problematic at the Sabino Canyon site, with significant models for only 0 and 7 days from present or 0, 7, and 14 days from present for the site-specific and all sites combined models, respectively. The Tumamoc Hill site had lower probabilities of agreement between observed and predicted values at 14 days from present than the Tucson Airport site, though these were still significant for the Tumamoc Hill site-specific model. The Sabino Canyon and Tumamoc sites are located on steep slopes with very shallow soils that likely experience frequent and dramatic fluctuations in soil moisture within the profile (Ehleringer and Cooper 1988; Turner et al. 2003; Yang and Lowe 1956). Conversely, the Tucson Airport site likely has the ability to store more soil moisture, and for longer time periods, allowing this site to rely less on individual pulses of precipitation. This is evident in the greater probability of correct prediction of greenness for the Tucson Airport vs. Sabino Canyon or Tumamoc Hill sites, especially at longer forecast time periods of 14, 21, and 28 days in the future (Table 1).

To elucidate the source of variation driving the models, we calculated standardized regression coefficients for each of the independent variables. With the exception of the Tucson Airport models, precipitation or temperature variables had the largest effect on predicted greenness up to 14 days into the future and day of year had the largest effect at 21 and 28 days into the future (Tables 2 to 5). Conversely, predicted greenness in the Tucson Airport models (Table 4) was most affected by temperature variables at 21 and 28 days into the future and day of year at 0 and 14 days into the future (the 7 day into the future model was most affected by mean 25-day relative humidity). For the all sites combined models (Table 2), mean 20-day relative humidity had the largest effect on predicted greenness up to 7 days in the future, while day of year had the largest effect on predicted greenness at 21 and 28 days into the future. For the Sabino Canyon models (Table 3), mean 20-day precipitation had the largest effect on predicted greenness up to 14 days in the future, and similar to the all sites combined models, day of year had the largest effect on predicted greenness at 21 and 28 days into the future. For the Tumamoc Hill models (Table 5), mean 25-day precipitation and mean 15-day minimum temperature had the largest effect on predicted greenness up to 14 days into the future, and again, day of year had the largest effect on greenness at 21 and 28 days into the future. These trends among sites indicate that the slope sites (Sabino Canyon and Tumamoc Hill) are more tightly coupled to weather variables in the near term than is the Tucson Airport site.

While successful in providing a means for managers to predict present and future buffelgrass greenness and therefore susceptibility to chemical control of this invader, the prediction capabilities of our models were constrained by several factors. First, with only two years of data, we took the most conservative approach of using Year 1 (2012) data to train the models and then tested them with Year 2 (2013) data. Additional years of testing data would allow us to combine the 2012 models with models based on both 2012 and 2013, likely encompassing additional seasonal variation in weather and the response of buffelgrass greenness and generation of more generalizable models. Further, in terms of seasonal precipitation, 2012 and 2013 were dramatically different for all sites, with 2013 being much drier and having fewer days above 60% greenness (Table 6). In 2012, Sabino

Canyon and Tumamoc Hill had 54 and 46 days above 60% greenness, respectively, while these same sites had only 20 and 9 days above 60% greenness in 2013. However, the Tucson Airport had 69 and 72 days above 60% greenness in 2012 and 2013, respectively, despite dramatically less summer rainfall in 2013. This corroborates the premise that runoff from adjacent sites was captured and its deeper soils supported a longer greenness response than at the slope sites. The antecedent weather variables that are partially responsible for affecting buffelgrass greenness likely had different effect magnitudes for 2012 versus 2013, at least for the slope sites of Sabino Canyon and Tumamoc Hill, and it's also possible that different weather variables were more important in each year. Buffelgrass greenness at these more xeric sites appear to be more strongly coupled to individual precipitation pulses, which are highly variable in space and time during the summer rainy season. Similar results for vegetation phenology have been found across a gradients of aridity (Ma et al. 2013). Given this variation, additional years or seasons of greenness and weather data would likely facilitate development of models with increased probabilities of correct prediction of buffelgrass greenness.

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Tables

Table 1: Probability (%) that predictions from multiple regression models based on 2012 data and observed greenness in buffelgrass from 2013 are simultaneously above or below the 60% threshold (a proxy for active vegetative growth and therefore susceptibility to glyphosate) for each of three study sites during the summer growing season.

	Sabino Canyon				Tucson Airport				Tumamoc Hill			
Days from present	% Site-specific	*P* Significance[a]	% All sites	*P* Significance	% Site-specific	*P* Significance	% All sites	*P* Significance	% Site-specific	*P* Significance	% All sites	*P* Significance
0	86 (78-92)[b]	<0.0001	88 (81-93)	<0.0001	81 (72-88)	<0.0001	86 (78-92)	<0.0001	76 (67-83)	<0.0001	78 (70-85)	<0.0001
7	83 (75-89)	<0.0001	80 (72-87)	<0.0001	90 (83-95)	<0.0001	89 (82-94)	<0.0001	78 (70-85)	<0.0001	82 (74-88)	<0.0001
14	61 (51-70)	0.0371	49 (40-59)	0.9212	92 (85-96)	<0.0001	93 (87-97)	<0.0001	59 (50-68)	0.0644	62 (53-71)	0.0148
21	54 (44-63)[b]	0.4884	60 (50-69)	0.0594	92 (85-96)	<0.0001	97 (92-99)	<0.0001	75 (66-83)	<0.0001	70 (59-76)	0.0003
28	59 (49-68)[b]	0.0918	60 (50-69)	0.0594	95 (89-98)	<0.0001	98 (93-99)	<0.0001	79 (71-86)	<0.0001	73 (63-80)	<0.0001

[a] One-sample exact binomial test, where agreement would occur with probability 0.5 if the model had no predictive value (i.e., the proportion of correct predictions of greenness such a sample does not significantly differ from the hypothesized value of 50%) (VassarStats: Website for Statistical Computation, Binomial Probability calculator with two-tail exact test, http://vassarstats.net/binomialX.html) (Table 1)

[b] 95% confidence interval for means in parentheses.

Table 2: Descriptive statistics for buffelgrass greenness models trained with 2012 data from all sites combined for 0, 7, 14, 21, and 28 days from present and tested individually on the Sabino Canyon, Tucson Airport, and Tumamoc Hill sites with 2013 observations.

Days in future	Parameter	Coefficient	Standardized Coefficient[a]	Parameter *F*	Model *F*	R^2 2012[b]	R^2 Sabino Canyon[bc]	R^2 Tucson Airport[bc]	R^2 Tumamoc Hill[bc]
0	Intercept	-229.67	NA	NA	218.34	0.78	0.41	0.71	0.24
	Day of year	0.30	0.40	79.12					
	Mean 20-day precipitation	0.32	0.43	150.27					
	Mean 15-day maximum temperature	3.85	0.32	55.99					
	Mean 20-day mean relative humidity	1.59	0.62	387.83					
	Site type	10.03	0.39	182.70					
7	Intercept	-160.08	NA	NA	194.86	0.72	0.16	0.79	0.24
	Mean 20-day precipitation	0.23	0.33	83.23					
	Mean 15-day maximum temperature	4.53	0.40	136.91					
	Mean 20-day mean relative humidity	1.14	0.48	182.85					

Days in future	Parameter	Coefficient	Standardized Coefficient[a]	Parameter F	Model F	R^2 2012[b]	R^2 Sabino Canyon[bc]	R^2 Tucson Airport[bc]	R^2 Tumamoc Hill[bc]
	Site type	10.21	0.42	181.39					
14	Intercept	-48.09	NA	NA	195.12	0.76	0.05	0.80	0.30
	Day of year	-0.28	-0.40	71.77					
	Mean 20-day precipitation	0.13	0.19	25.53					
	Mean 15-day maximum temperature	4.14	0.37	65.52					
	Mean 20-day mean relative humidity	0.49	0.21	40.11					
	Site type	9.78	0.41	182.56					
21	Intercept	47.88	NA	NA	299.34	0.79	0.15	0.84	0.55
	Day of year	-0.44	-0.60	188.79					
	Mean 20-day precipitation	0.09	0.13	17.68					
	Mean 15-day maximum temperature	3.10	0.26	39.16					
	Site type	8.51	0.34	143.97					
28	Intercept	111.35	NA	NA	258.14	0.77	0.07	0.85	0.50
	Day of year	-0.51	-0.68	222.65					

Days in future	Parameter	Coefficient	Standardized Coefficient[a]	Parameter *F*	Model *F*	R^2 2012[b]	R^2 Sabino Canyon[bc]	R^2 Tucson Airport[bc]	R^2 Tumamoc Hill[bc]
	Mean 20-day precipitation	0.10	0.13	17.29					
	Mean 15-day maximum temperature	1.70	0.14	11.47					
	Site type	7.00	0.28	90.00					

[a] Standardized by subtracting the mean and dividing by the standard deviation so that the coefficients represent the change in terms of standard deviations in the dependent variable that result from a change of one standard deviation in an independent variable (such as day of year, precipitation, temperature, relative humidity and site type variables).

[b] All R^2 are adjusted for the number of explanatory terms in a model relative to the number of data points.

[c] Observations from 2013.

Table 3: Descriptive statistics for Sabino Canyon buffelgrass greenness multiple regression models trained with 2012 data for 0, 7, 14, 21, and 28 days from present and tested with 2013 observations.

Days in future	Parameter	Coefficient	Standardized Coefficient[a]	Parameter F	Model F	R^2 2012[b]	R^2 2013[b]
0	Intercept	-169.27	NA	NA	393.92	0.94	0.56
	Day of year	0.37	0.43	35.11			
	Mean 20-day precipitation	0.42	0.63	254.20			
	Mean 15-day minimum temperature	3.00	0.25	13.19			
	Mean 25-day mean relative humidity	1.25	0.43	97.15			
7	Intercept	-51.03	NA	NA	150.40	0.81	0.22
	Mean 20-day precipitation	0.42	0.66	115.90			
	Mean 10-day maximum temperature	1.52	0.13	6.89			
	Mean 25-day mean relative humidity	0.77	0.28	25.20			
14	Intercept	26.03	NA	NA	168.12	0.87	0.04
	Day of year	-0.20	-0.25	5.57			
	Mean 20-day precipitation	0.38	0.59	104.14			
	Mean 15-day minimum temperature	3.60	0.32	9.77			
	Mean 25-day mean relative humidity	-0.39	-0.14	4.99			

	Intercept	219.86	NA	NA			
21	Day of year	-0.64	-0.75	430.49	348.73	0.91	0.05
	Mean 20-day precipitation	0.25	0.38	69.74			
	Mean 25-day mean relative humidity	-0.55	-0.19	23.36			
	Intercept	352.14	NA	NA			
	Day of year	-0.88	-1.02	184.26			
28	Mean 20-day precipitation	0.22	0.33	65.08	355.88	0.93	-0.01
	Mean 15-day minimum temperature	-3.50	-0.29	16.64			
	Mean 25-day mean relative humidity	-0.60	-0.21	21.26			

[a] Standardized by subtracting the mean and dividing by the standard deviation so that the coefficients represent the change in terms of standard deviations in the dependent variable that result from a change of one standard deviation in an independent variable (such as day of year, precipitation, temperature, relative humidity and site type variables).

[b] All R^2 are adjusted for the number of explanatory terms in a model relative to the number of data points.

Table 4: Descriptive statistics for Tucson Airport buffelgrass greenness multiple regression models trained with 2012 data for 0, 7, 14, 21, and 28 days from present and tested with 2013 observations.

Days in future	Parameter	Coefficient	Standardized Coefficient[a]	Parameter *F*	Model *F*	R^2 2012[b]	R^2 2013[b]
0	Intercept	-308.95	NA	NA	146.81	0.86	0.60
	Day of year	0.56	0.80	40.20			
	Mean 25-day precipitation	0.36	0.57	45.61			
	Mean 15-day mean temperature	5.42	0.51	29.53			
	Mean 25-day mean relative humidity	1.72	0.65	150.85			
7	Intercept	-66.59	NA	NA	154.27	0.87	0.75
	Day of year	-0.12	-0.20	6.62			
	Mean 10-day precipitation	0.18	0.20	16.73			
	Mean 15-day mean temperature	3.22	0.34	26.51			
	Mean 25-day mean relative humidity	1.58	0.69	326.56			
14	Intercept	184.83	NA	NA	173.68	0.85	0.85
	Day of year	-0.63	-1.02	308.71			
	Mean 25-day precipitation	-0.13	-0.23	13.70			
	Mean 25-day mean relative humidity	0.94	0.40	74.09			

Days in future	Parameter	Coefficient	Standardized Coefficient[a]	Parameter F	Model F	R^2 2012[b]	R^2 2013[b]
21	Intercept	-101.41	NA	NA	172.36	0.88	0.73
	Day of year	-0.15	-0.22	5.39			
	Mean 25-day precipitation	0.16	0.25	15.44			
	Mean 15-day mean temperature	6.46	0.55	48.43			
	Mean 5-day mean relative humidity	0.18	0.10	4.32			
28	Intercept	-98.47	NA	NA	238.79	0.88	0.70
	Day of year	-0.21	-0.27	8.77			
	Mean 25-day precipitation	0.22	0.30	26.96			
	Mean 15-day mean temperature	6.82	0.56	65.24			

[a] Standardized by subtracting the mean and dividing by the standard deviation so that the coefficients represent the change in terms of standard deviations in the dependent variable that result from a change of one standard deviation in an independent variable (such as day of year, precipitation, temperature, relative humidity and site type variables).

[b] All R^2 are adjusted for the number of explanatory terms in a model relative to the number of data points.

Table 5: Descriptive statistics for Tumamoc Hill buffelgrass greenness multiple regression models trained with 2012 data for 0, 7, 14, 21, and 28 days from present and tested with 2013 observations.

Days in future	Parameter	Coefficient	Standardized Coefficient[a]	Parameter *F*	Model *F*	R^2 2012[b]	R^2 2013[b]
0	Intercept	-4.79	NA	NA	140.00	0.72	0.53
	Mean 20-day precipitation	0.52	0.52	92.67			
	Mean 20-day mean relative humidity	1.05	0.50	85.64			
7	Intercept	-50.29	NA	NA	52.55	0.58	0.31
	Mean 5-day precipitation	0.81	0.42	40.45			
	Mean 15-day minimum temperature	3.83	0.45	47.73			
	Mean 20-day mean relative humidity	0.40	0.21	10.90			
14	Intercept	-7.36	NA	NA	81.96	0.69	0.26
	Day of year	-0.20	-0.33	11.98			
	Mean 20-day precipitation	-0.22	-0.24	14.44			
	Mean 15-day minimum temperature	5.23	0.64	44.65			
21	Intercept	105.16	NA	NA	122.19	0.77	0.58
	Day of year	-0.47	-0.77	85.13			
	Mean 20-day precipitation	-0.23	-0.25	20.56			

Days in future	Parameter	Coefficient	Standardized Coefficient[a]	Parameter F	Model F	R^2 2012[b]	R^2 2013[b]
	Mean 10-day mean temperature	2.16	0.25	11.24			
	Intercept	319.79	NA	NA			
	Day of year	-0.79	-1.35	157.01			
28	Mean 5-day precipitation	-0.35	-0.19	13.94	81.76	0.74	0.57
	Mean 15-day minimum temperature	-4.42	-0.55	26.75			
	Mean 20-day mean relative humidity	0.31	0.17	7.64			

[a] Standardized by subtracting the mean and dividing by the standard deviation so that the coefficients represent the change in terms of standard deviations in the dependent variable that result from a change of one standard deviation in an independent variable (such as day of year, precipitation, temperature, relative humidity and site type variables).

[b] All R^2 are adjusted for the number of explanatory terms in a model relative to the number of data points.

Table 6: Summer precipitation (June through October) of study sites in 2012 and 2013 compared to the long-term mean, and number of days that buffelgrass greenness was 60% or above.

Site	Long Term Mean[a]	2012			2013		
	mm	mm	%	day[b]	mm	%	day[b]
Sabino Canyon	216	241	111	54	144	67	20
Tucson Airport	179	153	86	69	95	53	72
Tumamoc Hill	171	154	95	46	112	69	9

[a] 1981-2010 for Sabino Canyon and the Tucson Airport (Western Region Climate Center, http://www.wrcc.dri.edu/summary/Climsmaz.html), 1984-2007 for Tumamoc Hill (University of Arizona).

[b] Days above 60% greenness.

Figures

Figure 1: Buffelgrass plants can fluctuate rapidly between active growth and dormancy during the summer rainy season.

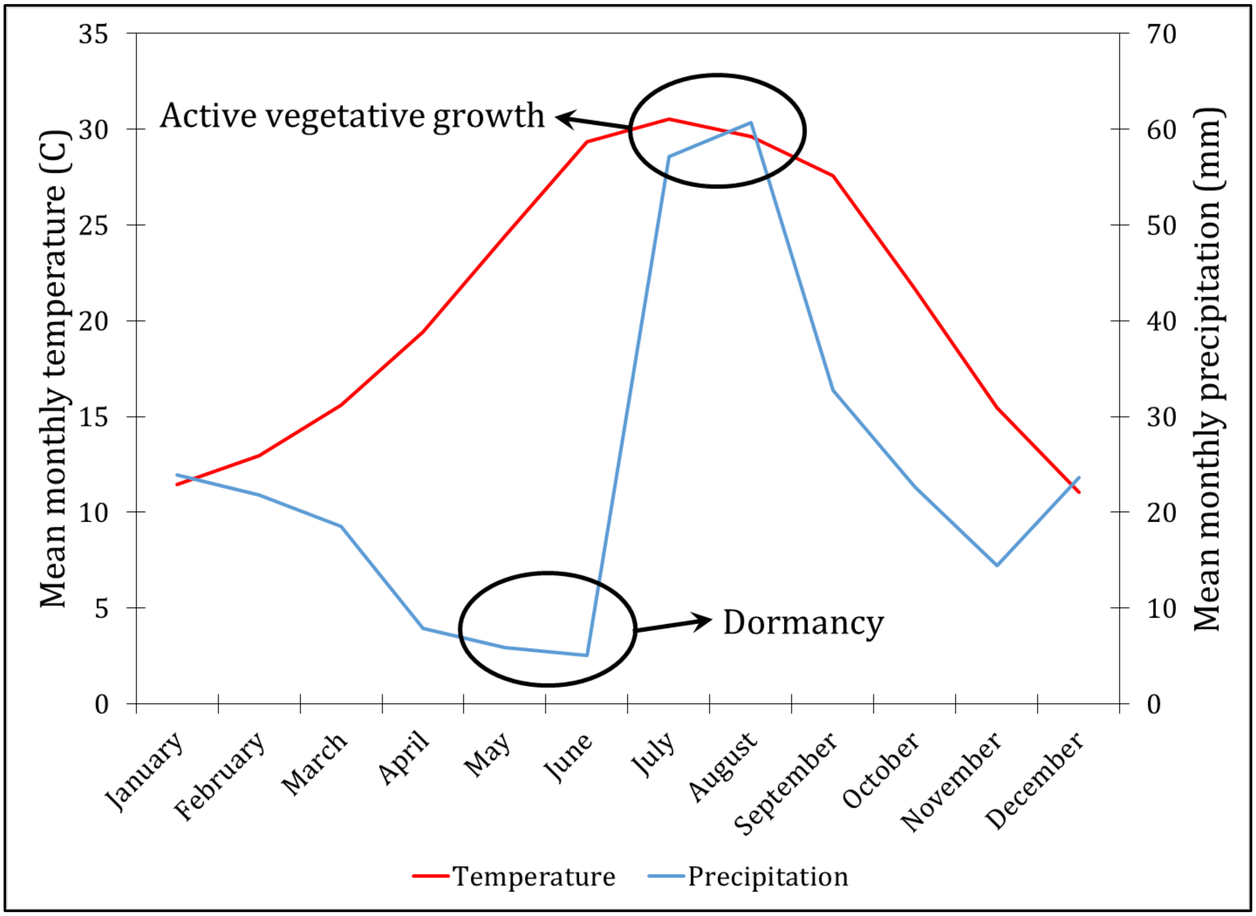

Figure 2: Seasonal predictability of buffelgrass phenology in the Sonoran Desert of Arizona. Plants will become dormant sometime during the May through June time frame, and will experience active vegetative growth during the July through August time frame. Phenology is difficult to predict during any other time frame.

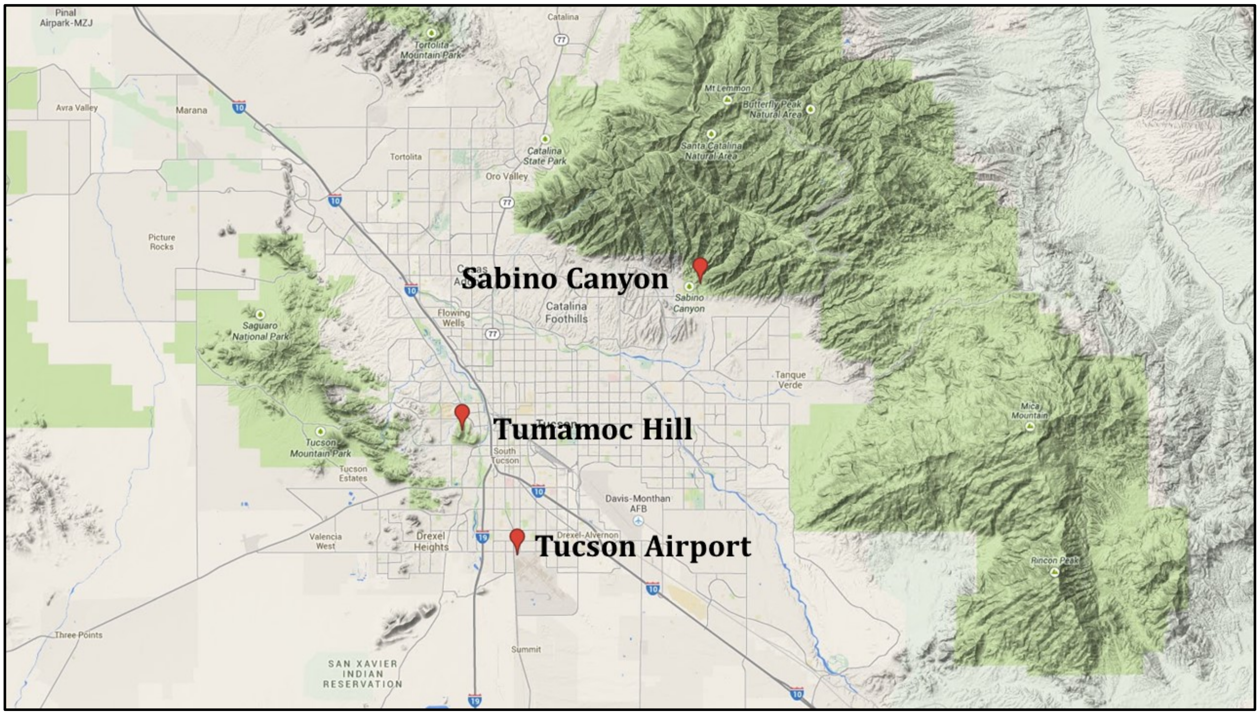

Figure 3: Relative locations of Sabino Canyon, Tumamoc Hill and Tucson Airport study sites.

Figure 4a: Sabino Canyon camera image and polygon from inside which red, green and blue digital numbers were extracted.

Figure 4b: Tucson Airport camera image and polygon from inside which red, green and blue digital numbers were extracted.

Figure 4c: Tumamoc Hill camera image and polygon from inside which red, green and blue digital numbers were extracted.

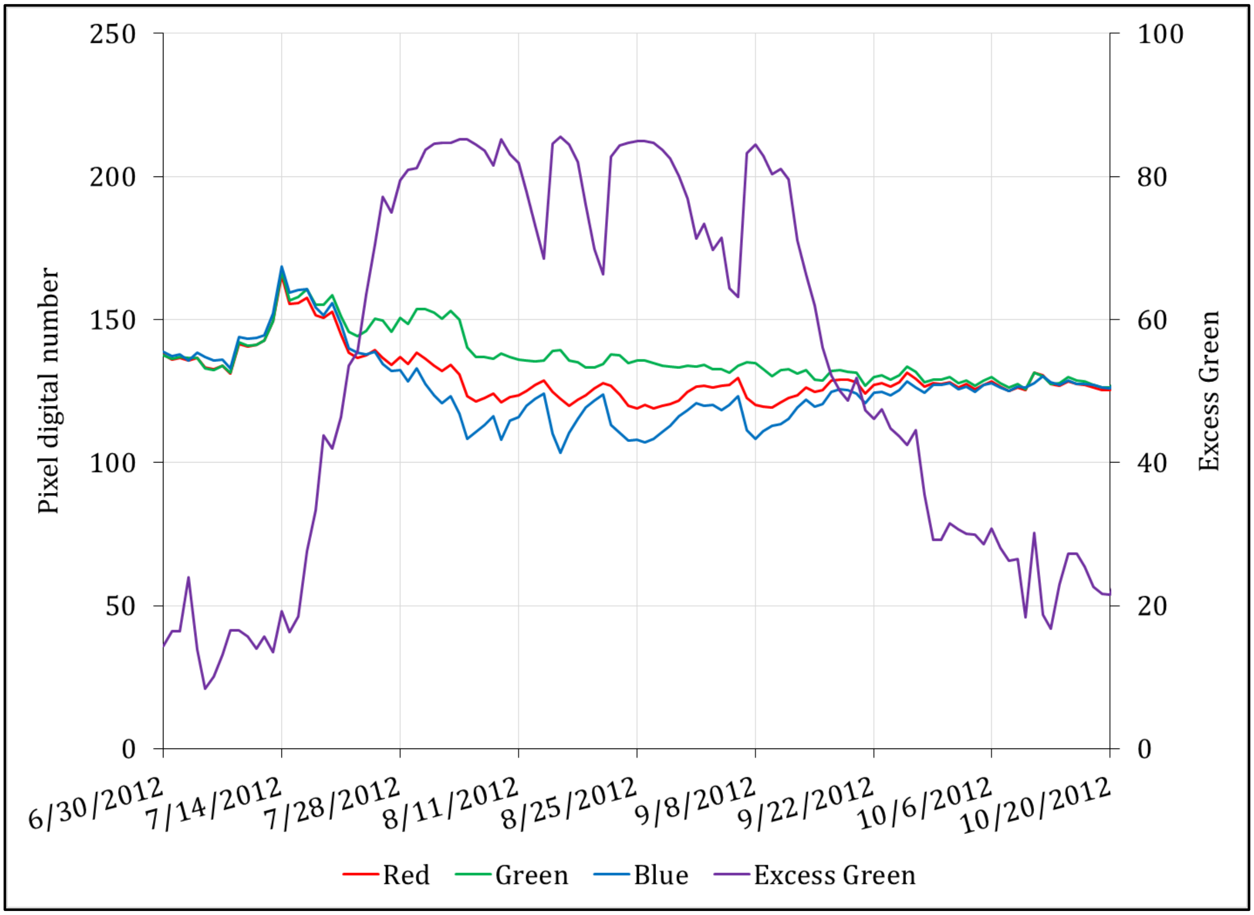

Figure 5a. Mean daily red, green, and blue pixel digital numbers and calculated Excess Green index (combined difference of green minus red and green minus blue pixel digital numbers) from analyzed images of buffelgrass captured at Sabino Canyon during summer 2012.

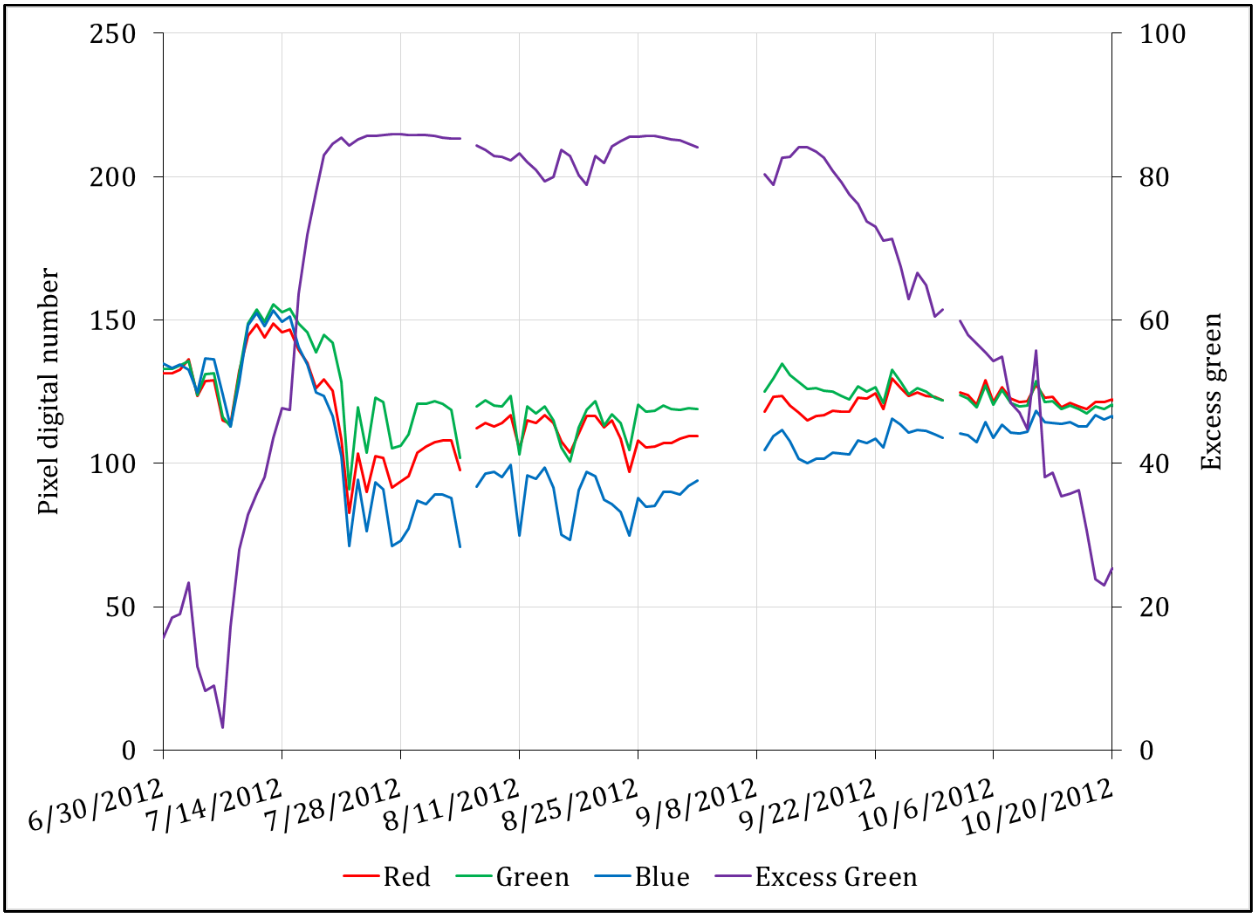

Figure 5b. Mean daily red, green, and blue pixel digital numbers and calculated Excess Green index (combined difference of green minus red and green minus blue pixel digital numbers) from analyzed images of buffelgrass captured at the Tucson Airport during summer 2012.

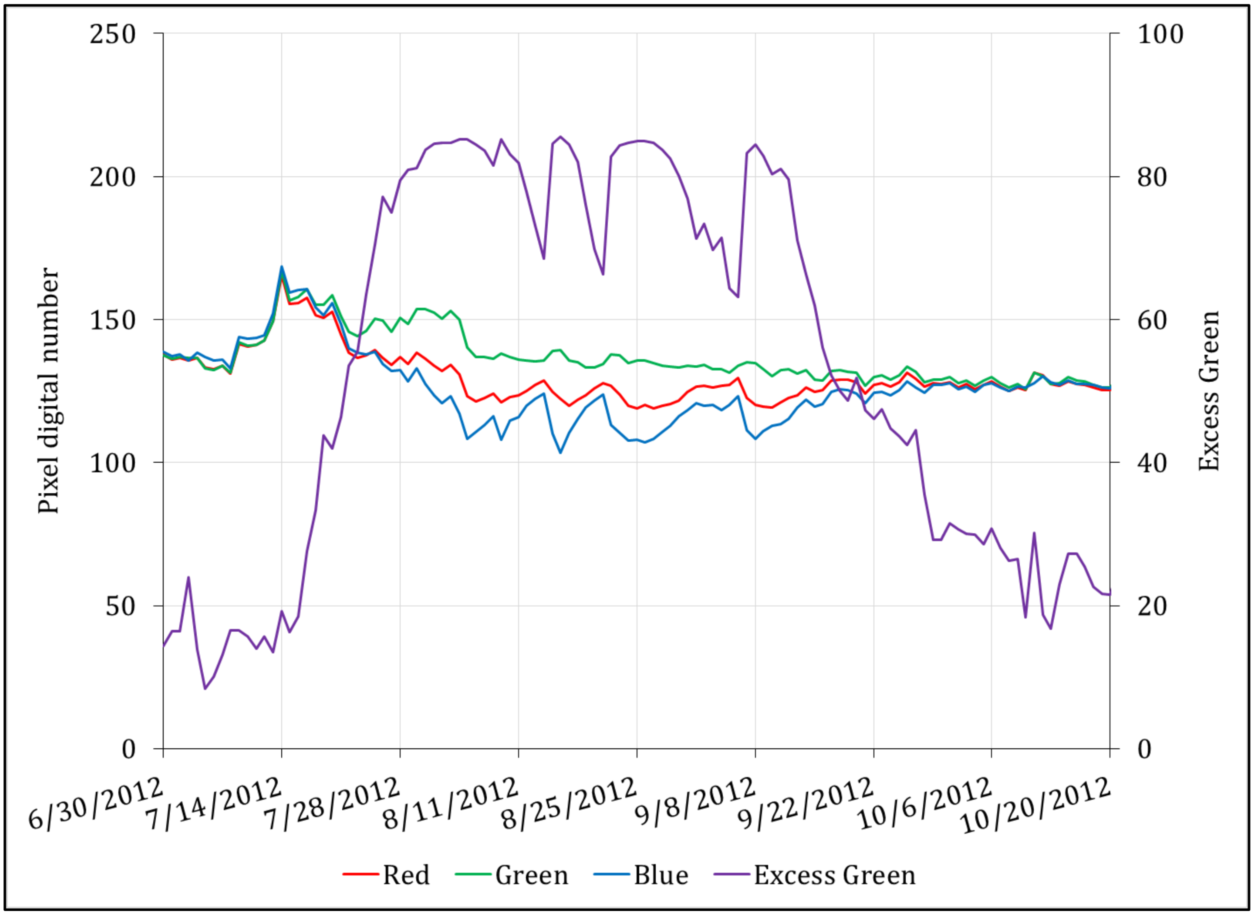

Figure 5c: Mean daily red, green, and blue pixel digital numbers and calculated Excess Green index (combined difference of green minus red and green minus blue pixel digital numbers) from analyzed images of buffelgrass captured at Tumamoc Hill during summer 2012.

Figure 6: Six approximate classes of visual greenness: 0 (a), 1 to 20 (b), 21 to 40 (c), 41 to 60 (d), 61 to 80 (e), and 81 to 100% (f) green.

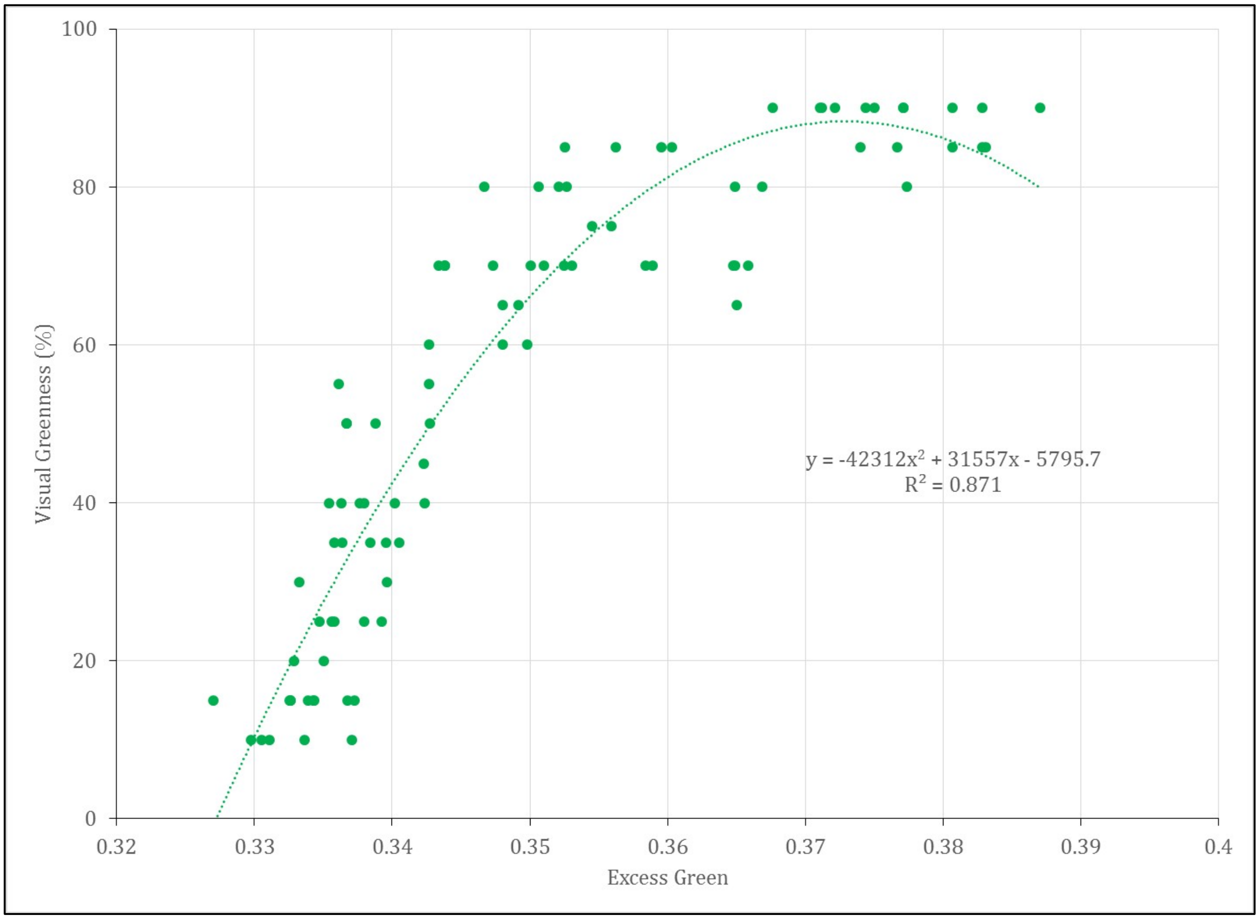

Figure 7. Regression of visually estimated buffelgrass image greenness on calculated Excess Green index (combined difference of green minus red and green minus blue pixel digital numbers) for 90 random images, stratified by site and camera.

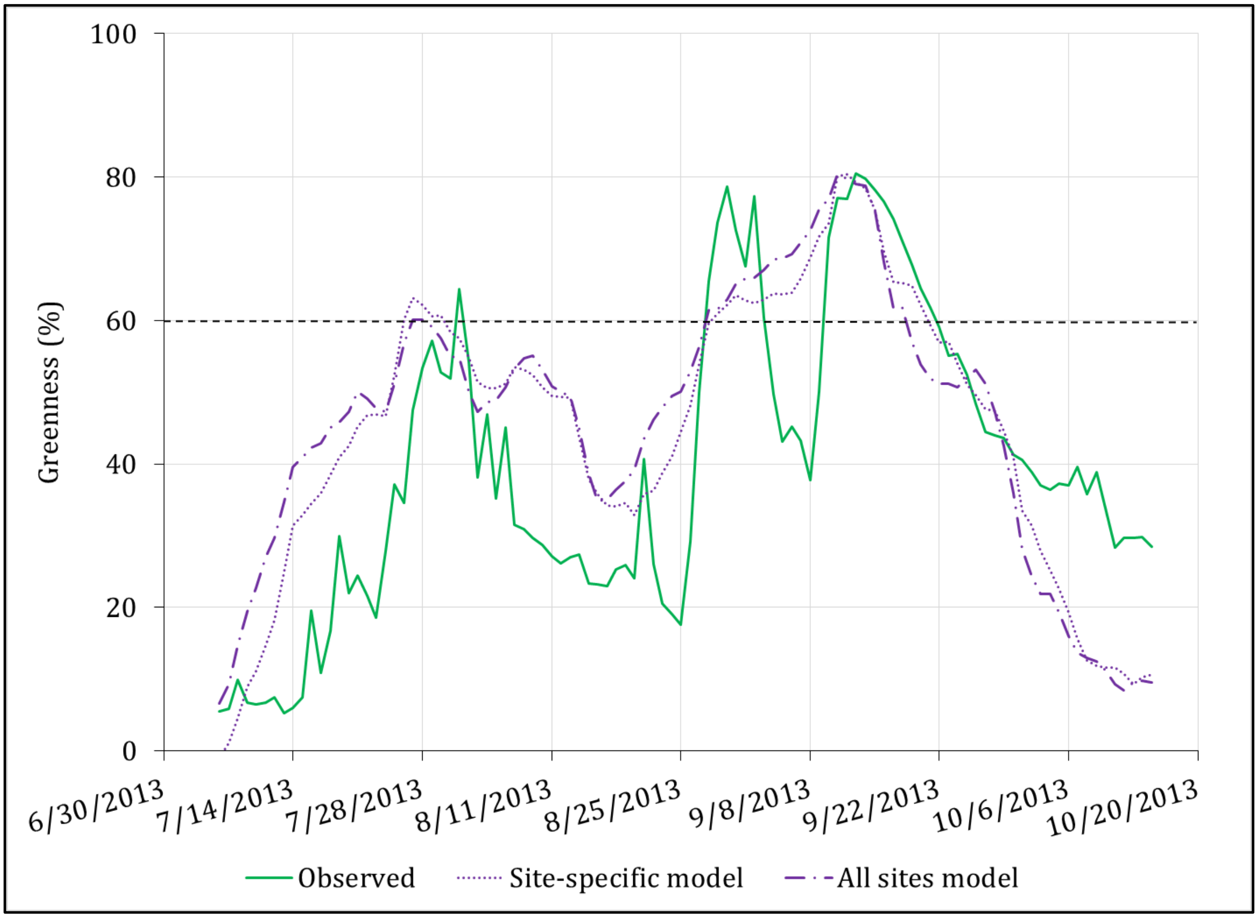

Figure 8a. Predicted (2012 Model) and observed (2013 data) buffelgrass greenness for Sabino Canyon during summer 2013, showing 60% greenness threshold for herbicide susceptibility.

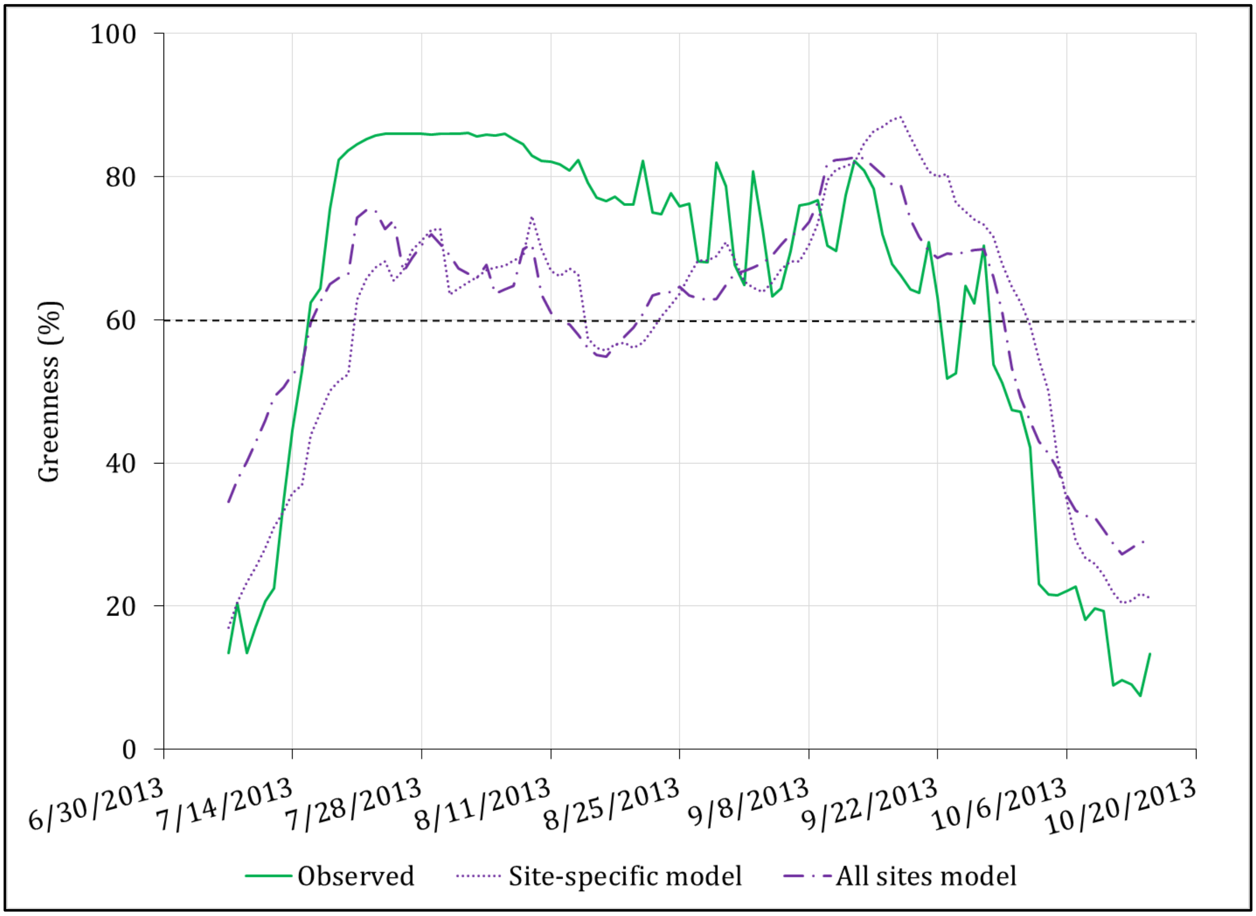

Figure 8b. Predicted (2012 Model) and observed (2013 data) buffelgrass greenness for the Tucson Airport during summer 2013, showing 60% greenness threshold for herbicide susceptibility.

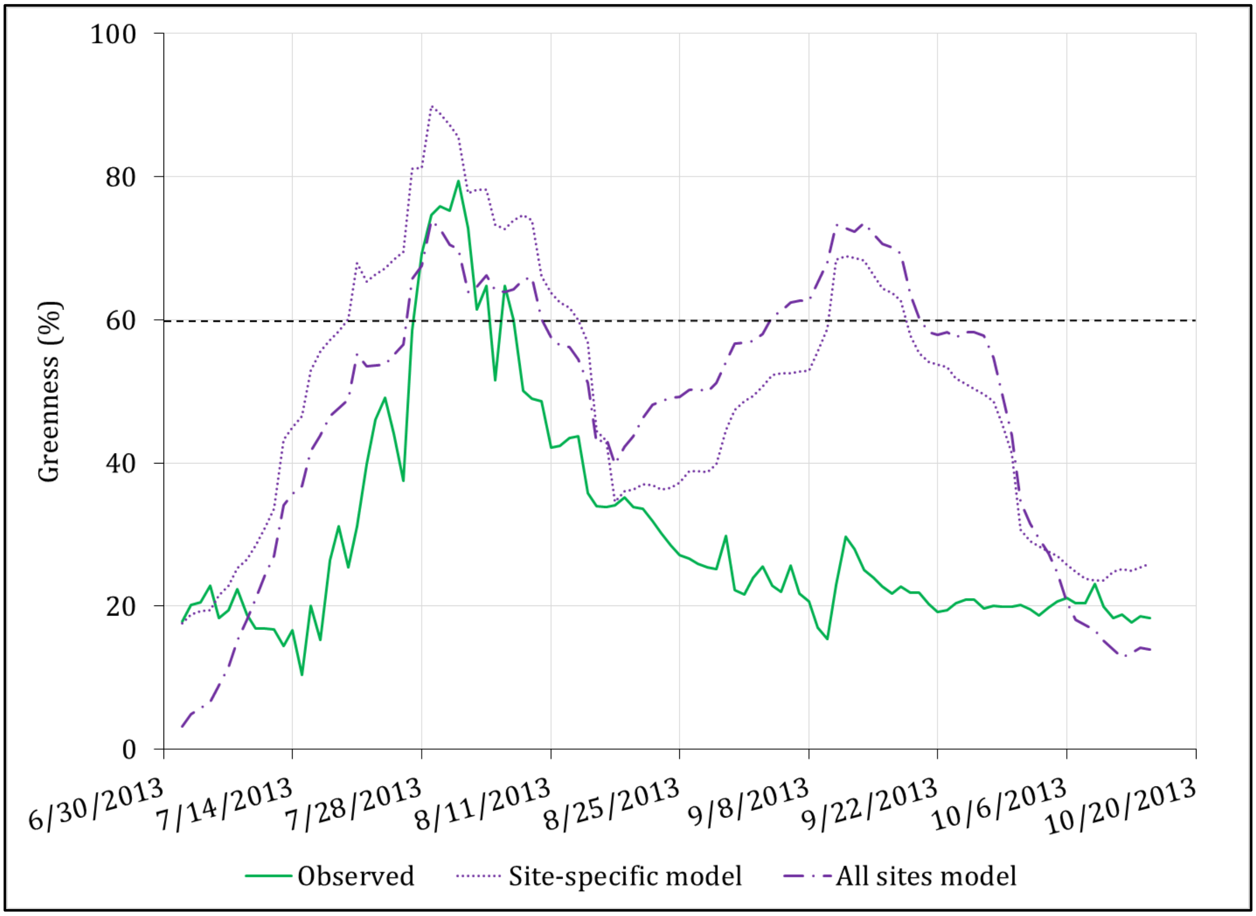

Figure 8c. Predicted (2012 Model) and observed (2013 data) buffelgrass greenness for Tumamoc Hill during summer 2013, showing 60% greenness threshold for herbicide susceptibility.

APPENDIX B: Improved chemical control of buffelgrass (*Cenchrus ciliaris*) in the Sonoran Desert

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Abstract

Buffelgrass is a perennial C_4 bunchgrass that can be invasive in subtropical regions worldwide. Current chemical control of buffelgrass in Arizona's Sonoran Desert primarily relies on glyphosate, which has no preemergence activity, is not selective for grasses, and cannot be applied when buffelgrass is dormant. To address these issues, we conducted a series of replicated field experiments from 2010 to 2013 at an undisturbed wildland site and a former agricultural field near Tucson, AZ to investigate herbicides and herbicide mixtures, application rates, and application timing effects for control of buffelgrass. We evaluated the effects of different rates of two herbicides (imazapic and clethodim), alone or in combination with different rates of glyphosate, for pre- (imazapic only) and postemergence control of buffelgrass. We also evaluated growing- and dormant-season (summer and winter, respectively) application of imazapyr for pre- and postemergence control of buffelgrass. We used CO_2 pressurized sprayers to apply herbicides at known pressures, speeds, and carrier rates to ensure accurate broadcast application rates. Our results indicate that when applied alone, glyphosate rates of 2.52 kg acid equivalent (ae) ha^{-1} are needed to kill mature plants in a single application. Lower rates of glyphosate were effective when combined with imazapic. Imazapic did not kill mature buffelgrass plants even at the highest label rate, although this rate (0.21 kg ae ha^{-1}) did suppress shoot growth at 36 months after treatment (MAT). Clethodim did not have any effect on buffelgrass, even at the highest label rate. At one of two sites, imazapyr killed mature buffelgrass plants 6 MAT when applied during the dormant season at 0.56 kg ae ha^{-1}, or 12 MAT when applied during the growing season at 1.12 kg ae ha^{-1}. Imazapyr provided effective preemergence control of buffelgrass at all rates 6 and 12 MAT. Thus, while glyphosate can kill mature buffelgrass plants after a single application, its utility is limited by the short and unpredictable growing season when buffelgrass is susceptible and by its lack of soil activity. Imazapyr shows promise for year-round pre- and postemergence control of buffelgrass.

Nomenclature: Clethodim; glyphosate; imazapic; imazapyr; buffelgrass, *Cenchrus ciliaris* L.

Key words: dormant season application, graminicide, herbicide tank mixtures, pre- and postemergence control, selectivity.

Management Implications:

Based on our results managers in the Sonoran Desert should use a broadcast rate of 2.52 kg ae ha^{-1} glyphosate to provide reliable postemergence mortality of established, green buffelgrass plants in a single application during the growing season. If imazapic is included at a rate of at least 0.07 kg ae ha^{-1}, a lower rate of glyphosate (1.68 kg ae ha^{-1}) may be effective. Lower glyphosate rates applied later in the growing season when more soil moisture has accumulated and temperatures are lower can also be effective, though the higher rate is currently recommended. Imazapic does not appear to kill mature buffelgrass plants, even at high rates (0.21 kg ae ha^{-1}), although it can suppress shoot growth for an extended (3-yr) period. In addition, a high rate (0.21 kg ae ha^{-1}) of imazapic suppressed buffelgrass seedling emergence up to 12 MAT. However, the high price of the imazapic may make the potential pre- and postemergence control benefits not cost effective. No rate of clethodim tested, including the maximum and double the maximum label rate, had any effect on postemergence buffelgrass response variables. Related studies with sethoxydim and fluazifop had similar results, indicating that mature buffelgrass plants are not susceptible to available graminicides under these experimental conditions. Imazapyr shows promise for pre- and postemergence buffelgrass control and killed mature buffelgrass plants at one study site 6 MAT when applied at 0.56 kg ha^{-1} during the dormant season and at 12 MAT when applied at 1.12 kg ha^{-1} during the growing season. Complete preemergence control of buffelgrass seedlings 6 and 12 MAT was achieved at the lower rate at this site for both application timings. At the other study site, plants were not killed, but did show signs of herbicide damage (leaf necrosis and yellowing) at all application rates and timings, indicating that the plants may still die over time. At this site complete preemergence control of buffelgrass seedlings 6 and 12 MAT was also achieved at the lowest rate for both application timings. In summary, glyphosate can kill mature buffelgrass plants after a single application, but its utility is limited by the short and unpredictable growing season of buffelgrass and by glyphosate’s lack of soil activity. Imazapyr shows promise for year-round pre- and postemergence control of buffelgrass.

Introduction

Buffelgrass is a perennial African bunchgrass that is rapidly spreading throughout parts of the Sonoran Desert through both deliberate planting (Franklin et al. 2006; Franklin and Molina-Freaner 2010) and invasion into undisturbed areas (Olsson et al. 2012b). Once established, it aggressively competes with and displaces native Sonoran Desert plant species (Eilts and Huxman 2013; Lyons et al. 2013; Morales-Romero et al. 2012), resulting in ecosystem replacement (Olsson et al. 2012a) and eventually the potential for altered fire regimes (McDonald and McPherson 2011; McDonald and McPherson 2013; Schlesinger et al. 2013). Further, although local populations likely have low genotypic diversity (Gutierrez-Ozuna et al. 2009), buffelgrass exhibits a high level of phenotypic plasticity allowing it to invade a range of arid environments (Arshad et al. 2007; Kharrat-Souissi et al. 2014; Mnif et al. 2005). As such, buffelgrass invades a variety of sites in the Sonoran Desert, and the efficacy of management approaches may be different at each due to differing phenologies and morphologies that can affect translocation of herbicides within the plant (Bussan and Dyer 1999; Devine et al. 1993; Ross and Lembi 2008).

Historically, weed management experiments with buffelgrass focused primarily on *avoiding* herbicide injury to buffelgrass being grown as a forage crop (Baur et al. 1977; Bovey et al. 1979; Bovey et al. 1980; Bovey et al. 1984; Bovey and Hussey 1991; Mayeux and Hamilton 1983; Rasmussen et al. 1986). In recent years, studies have begun to evaluate herbicide effects and effectiveness for use in managing buffelgrass invasions (studies focused on maximizing damage to buffelgrass). Australian land managers have examined the efficacy of several herbicides for controlling buffelgrass, including glyphosate, fluazifop and haloxyfop in Queensland (Dixon et al. 2003) and flupropanate in South Australia

(Biosecurity 2012). Otherwise these studies have been largely based in Arizona and Texas, perhaps because these two states have advanced invasions in high-value federal lands such as Saguaro and Big Bend National Parks and several other parks, reserves and forests. Of the herbicides evaluated, only glyphosate and imazapyr have been used successfully to kill mature plants in a single application (D. Backer, personal communication, "Herbicide efficacy trials on buffelgrass," 2009). In Arizona the managers typically report using hand-pump backpack sprayers and mix glyphosate and water at a 2-5% solution (D. Backer, National Park Service; S. Biedenbender, US Forest Service; D. Tersey, Bureau of Land Management; D. Siegel, Pima County; personal communication). Unfortunately, no calibration is used to standardize the amount of solution applied on a per area basis so the actual amount of herbicide applied per area unknown and probably highly variable (Alam and Hussain 2010; Spencer and Dent 1991). This is true for both on-the-ground herbicide application for buffelgrass control and in the previously mentioned herbicide evaluation studies on buffelgrass (D. Backer, personal communication, "Herbicide efficacy trials on buffelgrass," 2009).

The advantage of this technique for herbicide application is that it is simple and straightforward, requiring very little special training or calculations to mix and apply the herbicide (Millstein 1995). The disadvantage is that tank pressure and spraying times per plant can vary widely (especially when terrain and buffelgrass densities are not uniform, which they rarely are in wildland settings), resulting in a high range of herbicide being applied on a per area basis with potentially large differences in plant responses (Alam and Hussain 2010), and perhaps most importantly, no clear means for reproducibility of the treatments. To correct for these disadvantages, the alternative is to calibrate backpack sprayer applications for constant pressure, constant nozzle height, known spray swath areas or widths, carrier rates, and speeds of application (Alam and Hussain 2010; Dent and Spencer 1993). Such applications require more training, more calculations, and special equipment, which discourage managers from utilizing calibrated broadcast techniques. For foliar uptake herbicides like glyphosate with no soil activity (Baylis 2000) and little risk from over application, the disadvantages of broadcast outweigh the benefits in wildland settings. However, this has prevented any quantitative estimation of the actual amount of foliar uptake herbicide required to kill buffelgrass, so rates reported to be effective by managers are not comparable and offer little guidance for adjusting tank mixtures to suit different field conditions. Individual plant treatment, unless modified for calibration to regulate herbicide applied per area, may also be a risky technique if soil-mobile herbicides such as imazapyr prove to be effective for buffelgrass control.

Backer and Foster (2007) found that glyphosate was effective at killing buffelgrass plants in Arizona with a single application, and it has been the herbicide of choice among managers since active chemical control efforts began in Arizona, likely due to its effectiveness and low potential for non-target damage. However, reliance on glyphosate as the sole herbicide for chemical control of buffelgrass poses several potential problems for managers. First, glyphosate has no soil activity or effect on buffelgrass seeds in the soil that may remain viable for several years (Winkworth 1963; Winkworth 1971) and infested areas need to be retreated for long-term control. Second, glyphosate is non-selective and may damage some non-target species, depending on the amount of chemical exposure and treatment frequency. Third, glyphosate is symplastically translocated, requiring active vegetative growth for optimum action on meristematic tissue (Ross and Lembi 2008).

Potential limitations of glyphosate herbicide justify the exploration of alternative herbicides for chemical control of buffelgrass, and to ensure that the results of these studies can be utilized across a maximum number of settings, it is essential that they not be performed with the traditional IPT application methods.

The objectives of this research were to address the above concerns by systematically screening herbicides with potential for preemergence activity (imazapic and imazapyr), monocot selectivity (clethodim), and dormant-season application (imazapyr), alone or in combination with glyphosate to answer three questions. First, can imazapic applied alone or in mixture with glyphosate reduce repeated postemergence treatments by controlling seedling emergence or by improving glyphosate activity on existing plants? Second, can clethodim and glyphosate mixtures effectively control buffelgrass in order to reduce potential glyphosate damage to native non-target vegetation? Third, can imazapyr applied to dormant buffelgrass provide effective control in the subsequent growing season and thus extend the limited treatment opportunities occurring in July to September to the remainder of the year?

We implemented and monitored experiments at a site near Pusch Ridge in the Santa Catalina Mountains (site A) and near Robles Junction, AZ (site B) from 2010 to 2013 to address these objectives. To simplify discussion, studies are given reference codes in Table 1. Studies A1, A2, and B1 addressed the interaction of glyphosate and imazapic rates and their postemergence effects on mature plant mortality and preemergence suppression of buffelgrass seed germination. Studies A3, A4, B2, and B3 addressed the interaction of clethodim and glyphosate in providing more selective postemergence control of mature buffelgrass plants. Studies A5 and B4 address the ability of postemergence imazapyr applications during the dormant season to control mature buffelgrass plants during the subsequent growing season. Study B5 continues the evaluation of imazapic for preemergence control of buffelgrass seedling emergence and establishment.

Materials and Methods

Study sites. We established a total of ten experiments at two study sites (Figure 1) over four years; site locations, treatment dates, herbicides, herbicide rates, and replications per experiment are listed in Table 1. Trade names and concentrations of the herbicide products used are listed in Table 2. Permits for access and herbicide application were obtained in 2010 and again in 2012. Since 2010, over 1,000 plots were established, treated, measured (visually assessed greenness, basal diameter, number of actively growing tillers) pre- and post-herbicide application.

The first study site, Site A, (32.357407, -110.952955) was established in 2010 and is located at the western edge of the Santa Catalina Mountains on a southwest-facing slope near Pusch Ridge north of Tucson, AZ (Figure 2). This site has well-drained, shallow, very gravelly sandy loam soils or exposed bedrock (Cochran and Richardson 2003)with vegetation outside the infestation being typical of the Arizona Upland Subdivision of the Sonoran Desert and dominated by little-leaf paloverde (*Parkinsonia microphylla* Torr.) and saguaro (*Carnegiea gigantea* (Engelm.) Britton & Rose). Buffelgrass plants at this location appear to be typical of populations infesting undisturbed areas in the wildland-urban interface near Tucson. These plants rarely go completely dormant, having secondary branching of the tillers and usually keeping at least some green leaves during the winter months. Individual plants used for experiments had 37 to 39 tillers (counted at 10 cm [4 in] above soil level) and a basal diameter of 15 to 20 cm (6 to 8 in). (Tiller counts and basal diameters in site descriptions are estimated [95% confidence limits] for all plants measured at the site.)

The second study site, Site B, (32.068040, -111.333580) is located in a former agricultural field 43 km (27 mi) west of Tucson near the town of Robles Junction, AZ (Figure 3). The topography is flat with an extremely gravelly sandy loam soil that is deeper than Site A (51 to 102 cm Vs. 0 to 51 cm [20 to 40 in Vs. 0 to 20 in]) (Cochran and Richardson 2003), and the surrounding vegetation is dominated by velvet mesquite (*Prosopis velutina* Wooton). The fields were seeded to buffelgrass in the 1980s by University of Arizona Cooperative Extension and the Agricultural Research Service for reclamation purposes (Cox and Thacker 1992; Cox and Thacker 1996). Buffelgrass populations are most dense at the lowest ends of the fields, which are subject to occasional flooding and receive supplemental moisture from surface runoff during precipitation events (T. Bean, personal observation). Buffelgrass plants here are more typical of populations found in disturbed areas such as roadsides, ephemeral watercourses, and core urban areas of Tucson. Individual plants are larger than the plants found in undisturbed areas on slopes, having 52 to 102 tillers per plant and basal diameters of 18 to 26 cm (7 to 10 in). Possibly due to their location on the valley floor, these plants may be subject to cold air drainage and tend to go completely dormant in the winter months (T. Bean, personal observation). It is also rare to find secondary branching of the tillers at Site B, which instead usually produce new tillers only from the base of the plant at soil level.

Experimental design. All herbicide experiments (Table 1) used a randomized complete block design; the experimental unit was a single plant or 1 plant per plot. Where two herbicides were studied including tank mixtures, treatments represented the full factorial of combinations. Plots were installed in early summer of each application year (Table 1). Each experiment consisted of replicates immediately adjacent to each other where all experimental conditions were repeated in a randomized complete block design. Each replication was established by placing a 50-m (170 ft) tape along the contour of the hill slope. At Site B replications were established perpendicular to the slope of the field. One end of each replication was marked with a rebar stake to allow relocation of transects. Mature plants were distinguished by the presence of dead or dormant tissue from previous growing seasons, the presence of perennial tillers, and plants greater than 20 cm (8 in) in height. Plots were centered on mature buffelgrass plants at least 3 m (10 ft) apart (to prevent overspray from adjacent plots) and were marked with a numbered, wire stake flag in each replication. One additional plant per replication was marked to allow for problems that might arise such as application errors within a replication or a premature death or loss of plant vigor before treatments were applied. Replications were placed parallel to each other following the slope and at least 3 m (10 ft) apart, with the process being repeated until all replications were installed. For both replications and plots, rejection criteria were used to ensure uniform buffelgrass density, and similar site characteristics like slope and slope aspect. These criteria dictated that all plots within a transect be on the same slope contour and elevation and that plots and replicates be located in the same contiguous buffelgrass patch.

Pre-treatment inventories were performed one day prior to treatment application. For each marked plant (plot), visually estimated buffelgrass greenness (%) was measured as a proxy for growth and plant vigor. Buffelgrass greenness was estimated throughout using 6 classes representing a gradient of percent green to dead or decadent material (0%, 1-20%, 21-40%, 41-60%, 61-80%, 81-100%). Except where noted, we also counted the number of live tillers per plant. Tillers were designated as live if they had green leaf lamina extending from any of the meristem nodes or if the bottom internodes were green. Live tillers were also limber while dead tillers were brittle and snapped of when forced to bend. Post-treatment monitoring initially took place in fall or spring following application in 2010, though best results were obtained in the summer or fall at least one year following application and we moved to this schedule after 2011. For response variables of visually estimated greenness and tillers, plants (treated and controls) were considered dead (visually estimated greenness or tillers = 0) if there was no visible green material, including an inspection on basal tiller internodes.

We used CO_2 pressurized backpack sprayers and 4-nozzle booms equipped with Tee-Jet XR8002 nozzles spaced 50 cm (20 in) apart for a total spray swath of 1.9 m (6.3 ft). The sprayers were calibrated to deliver a carrier volume of approximately 190 L ha^{-1} (20 gal ac^{-1}) at 140 kPa (20 psi). Herbicides were mixed on site and applications were made as early in the morning as possible to avoid the highest temperatures of the day. Applications were made by centering the spray boom over the marked plant (total area of herbicide application was approximately 2 by 2 m [7 by 7 ft]) and walking at a calibrated pace (3.2 kph [2 mph]) using a metronome to time steps. Each herbicide treatment also included 1% v/v methylated seed oil and 1% w/w ammonium sulfate. Controls were sprayed with a mixture of water, ammonium sulfate, and methylated seed oil.

Data were analyzed using the Proc Mixed procedure in SAS (SAS 2012, Version 9.3; SAS Institute, Inc., 100 SAS Campus Drive, Cary, NC 27513), with replications considered random effects and herbicide rates and their interaction being fixed effects. Where applicable, season of application (imazapyr experiments) was considered a fixed effect. Response variables include greenness, post-spray counts of green tillers (counted at approximately 5 cm [3 in] above ground level), and seedling counts within a 1-m (3 ft) radius of the sprayed plant. In summer 2012, we mowed and rototilled buffelgrass stands that were not previously treated at Site B in an attempt to encourage seedling emergence, which was not previously observed at either site in 2010 or 2011. We sprayed resprouting mature buffelgrass plants with glyphosate and installed replicated treatments in 3 by 3 m (10 by 10 ft) plots to evaluate imazapic rates for pre emergence control. After receiving rainfall on the site, seedlings were counted within a 1-m (3 ft) belt transect subsample of each plot (as opposed to the single-plant plots for all other studies). For all studies, we have used the most recent data (2013) in statistical analysis. Thus, we will discuss data collected 36, 24, 12 and 6 months after treatment (MAT), depending on the when herbicide was applied in each study (Table 1). To meet assumptions of normality, visually estimated greenness (percentage data) were arcsine transformed prior to analysis (Gomez and Gomez 1984). Live tillers counts were square-root transformed prior to analysis (Gomez and Gomez 1984). Similarly, seedling counts were square-root transformed prior to analysis. Seedlings were only observed at Site B and only in 2013, so these response data are reported only where applicable. Because of the relatively large number of treatment comparisons being made in most experiments, Tukey’s HSD (honest significant difference) was used for mean comparisons and back-transformed least squares (LS) means are reported. Buffelgrass seedlings within 1 m (3 ft) of the target plant were counted when the experiment was being conducted to determine preemergence effects (imazapic and imazapyr experiments). Experiments were not sprayed until mean pre-spray greenness was greater than 60% to ensure optimal conditions for herbicide uptake and translocation and to mimic conditions under which managers would be applying symplastically translocated herbicides. Where LS means for response variables (greenness or live tiller counts) were not significantly different than zero according to a t-test, treatments were considered to have resulted in complete mortality. To determine statistical significance a *P value* of ≤ 0.05 was used in all analyses.

Results and Discussion

Imazapic and Glyphosate. Mature buffelgrass plants can be killed by a single application of glyphosate at 2.52 kg ae ha^{-1} (2.25 lb ae ac^{-1}) or at 1.68 kg ae ha^{-1} (1.75 lb ae ac^{-1}) if mixed with imazapic at a rate of at least 0.07 kg ae ha^{-1} (0.06 lb ae ac^{-1}) (Table 3; Figure 4). However, rates as low as 0.84 kg ae ha^{-1} (0.75 lb ae ac^{-1}) glyphosate were effective in killing mature buffelgrass plants in study A3 (2011 application of clethodim and glyphosate; Table 4). Plants sprayed in both project years were located in adjacent stands of buffelgrass at the same site. Plants sprayed in 2010 were greener and smaller at the time of spraying than plants sprayed in 2011 (74 to 78% vs. 65 to 70% green, 14 to 16 vs. 18 to 23 cm [5.6 to 6.4 vs 7.2 to 9.2 in] basal diameter [95% confidence intervals for mean greenness and diameter]). Plants experiencing more active vegetative growth are more likely to be experiencing optimal herbicide uptake and translocation than are plants that are growing more slowly or experiencing environmental stresses like drought (Devine et al. 1993; Ross and Lembi 2008).One explanation for this difference in mortality among treatments may be that the smaller size and more exposed location of A1 plants caused them to be more conservative in their growth therefore translocating less herbicide to meristematic regions of the plant. These plants may also have been more likely to senesce under drought conditions, and if such conditions followed treatment applications, herbicide translocation would have been similarly limited. Plants were sprayed more than one month earlier in A1 than A3 (August 14, 2010 vs. September 21, 2011). Temperatures are usually cooler in September than immediately following the onset of the summer rainy season in July or August and plants may be under less water stress. Also, with greater accumulated precipitation, plants may have been growing more actively due to the less stressful conditions and therefore more able to actively translocate herbicide. Differences in the minimum glyphosate rate required to kill mature buffelgrass plants ranged from 0.56 (Table 9) to 2.52 kg ae ha^{-1} (Table 3) and were found across study sites, years, and experiments. This suggests more research is needed into the effects of plant size, landscape position, phenological activity, and antecedent weather conditions on herbicide activity.

For the 2011 imazapic and glyphosate trials at both sites (A2 and B1), herbicide rates were chosen after analyzing the initial results of the 2010 Site-A experiment in late March 2011, approximately 8 MAT. At that time, buffelgrass plants were green and actively growing due to a warm, wet spring, and little to no effects from previous herbicide treatments were observed. In response to these seemingly discouraging results, we increased the herbicide rates for A2 and B1 above those applied for A1 to ensure that measurable effects were observed. Unfortunately, buffelgrass plants often require an extended (one to two yr or more) to fully express the effects of herbicide treatments (Bean and McCloskey, this appendix). As a result, our minimum glyphosate rate (2.52 kg ae/ha) resulted in the death of all plants treated in the 2011 imazapic and glyphosate experiments at both sites (Tables 5 and 6). We applied the maximum label rate and twice the maximum label rate of imazapic in these experiments. When applied without glyphosate, both levels of imazapic significantly reduced tiller counts and greenness relative to controls 24 MAT at Site A, and at Site B, both rates resulted in complete plant mortality. We did not monitor adjacent buffelgrass or non-target plants that may have received overspray. Similarly, results from A1 show the 0.21 kg ae ha^{-1} (0.19 lb ae ac^{-1}) rate of imazapic alone provided significant suppression of buffelgrass growth 36 MAT (Table 3; Figure 4) compared to the control and lower imazapic rates applied without glyphosate, indicating that imazapic may be a useful tool for managers.

Seedling emergence at either site was not observed from 2010 through 2012, which prevented evaluation of preemergence effects of the imazapic treatments. To stimulate seedling emergence, in summer 2012, we mowed and rototilled buffelgrass stands and then installed replicated 3 by 3 m (10 by 10 ft) plots (not single-plant plots as in other studies) in study B4; the treatments included two rates of imazapic (Table 1). Serendipitously, in 2013, a substantial amount of seedling emergence was measured at Site B, although not at Site A. No preemergence effects were found in the B1 (2011 imazapic and glyphosate experiment) at Site B (Table 5), suggesting that if buffelgrass preemergence effects exist for imazapic, they do not last 24 MAT. In the B4 study (2012 imazapic application) the 0.21 kg ae ha^{-1} imazapic rates had LS mean seedling counts 12 MAT significantly different than the control (13 Vs. 61 seedlings). Imazapic has preemergence activity on buffelgrass, but only at the maximum label rate that lasts 12 to 24 MAT.

Clethodim and glyphosate. For both sites and years of application, neither clethodim rate nor its interaction with glyphosate rate had significant effects on any of the response variables measured, suggesting that clethodim is rarely effective at controlling buffelgrass, either alone or in combination with glyphosate (Tables 7, 8, and 9; Figure 5). Our experiments included rates as high as double the maximum label rate (0.56 kg ae ha^{-1} [0.50 lb ae ac^{-1}]). Similar results for fluazifop suggest that graminicides labeled for application in wildland settings are ineffective at providing consistent selective control of buffelgrass (W. M. McCloskey, unpublished data). The only significant effect observed in any of the clethodim experiments (A3, A4, B2, and B3) was associated with glyphosate rate. Minimum glyphosate rate sufficient to kill mature buffelgrass plants in a single application varied by experiment (Tables 4 and 7 to 9; Figure 5), and possible reasons for this are discussed above.

Imazapyr. In study A5, imazapyr was applied in July 2012 and February 2013. Both herbicide rate and season of application demonstrated significant effects for the response variables tested at 6 and 12 MAT. At rates of 1.12 kg ae ha^{-1} (1.00 lb ae ac^{-1}) imazapyr or higher, mortality could be expected regardless of season (Table 10). For the winter application, mortality 6 MAT resulted from the lowest applied rate of 0.56 kg ae ha^{-1} (0.50 lb ae ac^{-1}) imazapyr (Table 10; Figure 6). As previously stated, no seedling emergence was observed at Site A, so preemergence effects of imazapyr could not be evaluated at this site.

In study B4, buffelgrass seedling emergence was almost completely suppressed at 6 and 12 MAT for all rates of imazapyr and season of application tested (Table 11). Neither the season of application nor imazapyr rate significantly affected tiller counts although imazapyr rate did significantly affect visually estimated greenness 6 and 12 MAT (Table 11). This may be due to the larger plant size at Site B compared to plants at Site A, as more time may be required for herbicide to be translocated throughout the larger plants' meristematic tissue (Ross and Lembi 2008). However, Site B received only 101 mm (4.0 in) of July to September precipitation, compared to 150 mm (5.9 in) at Site A, which may have limited plant growth and herbicide uptake from the soil and leaf tissue at Site B. Given lags in measurable plant damage observed in other studies discussed here, it is possible that additional time and moisture since herbicide application may show results similar to those observed at Site A. This hypothesis is supported by a ballistic herbicide study (i.e., herbicides applied via paintballs) at Site B in 2010, in which imazapyr was applied to adjacent plants at much higher rates than used in this study, and plants still took more than one year to show evidence of herbicide damage and many plants were killed (J. Leary, T. M. Bean, and W. M. McCloskey, unpublished data).

Management recommendations. Based on the results of these experiments, we recommend that for buffelgrass populations that contain potentially susceptible native non-target vegetation, glyphosate be applied during periods of active vegetative growth at 2.52 kg ae ha^{-1}. Applications should be made as early in the morning as possible to avoid the highest temperatures of the day. Lower rates may be effective under specific circumstances that have not been fully investigated (plant size, rate of growth, phenological state, etc.), but the 2.52 kg ae ha^{-1} rate consistently killed mature plants in a single application when applied during periods of active growth. Repeat applications should be made in consecutive periods of active growth to reduce reinvasion from the soil seed bank. Imazapic can be tank mixed with glyphosate for increased control and possible preemergence effects, but it is costly and can be difficult to obtain in Arizona. Clethodim is not recommended for control of mature buffelgrass plants. If buffelgrass populations are monocultures and the risk of downslope damage to non-targets through soil movement is low, then we recommend that single applications of imazapyr at 0.56 kg ae ha^{-1} be used to kill mature plants and provide preemergence control of the seedbank during periods of dormancy or 1.12 kg ae ha^{-1} during periods of active growth. Imazapyr should only be applied with equipment calibrated to deliver constant pressure, constant nozzle height, known spray swath areas or widths, carrier rates, and speeds of application.

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Tables

Table 1: Locations of buffelgrass herbicide experiments, application dates and the rates applied at a hill slope site in the Santa Catalina Mountains (Pusch Ridge) and a basin floor site on former agricultural land (Robles Junction), both near Tucson, AZ.

Application date	Herbicides[a]	Rates	Replications	Treatments	Study code
		kg ae/ha		no.	
8/14/2010	imazapic glyphosate	0, 0.70, 0.14, 0.21 0, 0.84, 1.68, 2.52	12	16	A1
9/21/2011	imazapic glyphosate	0, 0.21, 0.42 0, 2.52, 3.36, 4.20, 5.04	12	15	A2
9/21/2011	clethodim glyphosate	0, 0.18, 0.28 0, 0.84	8	6	A3
7/26/2012	clethodim glyphosate	0, 0.28, 0.56 0, 0.63, 1.25	10	9	A4
7/26/2012 2/14/2013	imazapyr imazapyr	0, 0.56, 1.12, 1.68 0, 0.56, 1.12, 1.68	10	8	A5
8/26/2011	imazapic glyphosate	0, 0.21, 0.42 0, 2.52, 4.20	8	9	B1
8/26/2011	clethodim glyphosate	0, 0.18, 0.28 0, 0.63, 0.84	8	9	B2

Application date	Herbicides[a]	Rates	Replications	Treatments	Study code
9/20/2012	clethodim	0, 0.25, 0.56	8	9	B3
	glyphosate	0, 0.63, 1.25			
8/13/2012	imazapic	0, 0.70, 0.14, 0.21	10	40	B4
9/20/2012	imazapyr	0, 0.56, 1.12	8	6	B5
3/1/2013	imazapyr	0, 0.56, 1.12			

[a] All trials used a carrier volume of approximately 190 L ha^{-1}, methylated seed oil at 1% v/v and ammonium sulfate at 1% w/w.

Table 2: Trade names and concentrations of herbicide formulations used in various experiments.

Product	Active ingredient	Concentration
		g ae/L
Kleenup Pro®[a]	glyphosate	356
Aquamaster®	glyphosate	480
Plateau®	imazapic	240
Arrow 2 EC®	clethodim	240
Arsenal®	imazapyr	240

[a] Only used in the A1 study (2010 glyphosate and imazapic experiment).

Table 3: Effect of glyphosate and imazapic applied in August 2010 on buffelgrass tiller number and greenness at the Pusch Ridge site. Data are least squares means for data collected 36 MAT.

Glyphosate rate	Imazapic rate	LS mean tillers[a]	LS mean greenness[b]
kg ae/ha	kg ae/ha	no. tillers/plant	%
0	0	37.7 a[c]	60.0 a
0	0.70	23.8 ab	43.4 ab
0	0.14	25.8 ab	40.9 abc
0	0.21	7.2 bcd	11.7 cde
0.84	0	10.6 bcd	16.8 bcde
0.84	0.70	4.0 cd[d]	6.8 de[d]
0.84	0.14	18.4 abc	31.7 abcd
0.84	0.21	8.7 bcd	20.1 bcde
1.68	0	11.3 bcd	20.9 bcde
1.68	0.70	3.1 cd[d]	5.1 de[d]
1.68	0.14	0 d[d]	0.1 e[d]
1.68	0.21	2.5 cd[d]	3.4 de[d]
2.52	0	0 d[d]	0.1 e[d]
2.52	0.70	0 d[d]	0.1 e[d]
2.52	0.14	3.2 cd[d]	6.0 de[d]
2.52	0.21	3.2 cd[d]	6.0 de[d]

[a] CV = 80% for tillers.

[b] CV = 100% for greenness.

[c] Tukey's HSD used to calculate means separations. Values within a column with same letters are not different ($P \leq 0.05$).

[d] Not significantly different than 0 ($P \leq 0.05$) according to t-test performed on least squares mean.

Table 4: Effect of glyphosate and clethodim applied in September on buffelgrass tiller number and greenness at the Pusch Ridge site. Data are least squares means for data collected 24 MAT.

Glyphosate rate	Clethodim rate	LS mean tillers[a]	LS mean greenness[b]
kg ae/ha	kg ae/ha	no. tillers/plant	%
0	0	26.5 a[c]	47.5 a
0	0.18	27.9 a	52.5 a
0	0.28	26.8 a	52.5 a
0.84	0	0.1 b[d]	1.7 b[d]
0.84	0.18	0.5 b[d]	1.7 b[d]
0.84	0.28	1.9 b[d]	10.4 b[d]

[a] CV = 20% for tillers.

[b] CV = 40% for greenness.

[c] Tukey's HSD used to calculate means separations. Values within a column with same letters are not different ($P \leq 0.05$).

[d] Not significantly different than 0 ($P \leq 0.05$) according to t-test performed on least squares mean.

Table 5: Effect of glyphosate and imazapic applied in August 2011 on buffelgrass tiller number and greenness at the Robles Junction site. Data are least squares means for data collected 24 MAT.

Glyphosate rate	Imazapic rate	LS mean tillers[a]	LS mean greenness[b]	LS mean seedlings[c]
kg ae/ha	kg ae/ha	no. tillers/plant	%	no. seedlings[d]
0	0	50.3 a[e]	48.8 a	4.8 a[f]
0	0.21	4.4 b[f]	4.0 b[f]	11.8 a
0	0.42	0 b[f]	0.3 b[f]	9.0 a
2.52	0	0.3 b[f]	1.5 b[f]	13.0 a
2.52	0.21	0 b[f]	0.3 b[f]	5.4 a[f]
2.52	0.42	0 b[f]	0.3 b[f]	13.1 a
4.20	0	5.1 b[f]	5.2 b[f]	4.0 a[f]
4.20	0.21	0 b[f]	0.3 b[f]	4.9 a[f]
4.20	0.42	0 b[f]	0.3 b[f]	8.0 a[f]

[a] CV = 80% for tillers.

[b] CV = 90% for greenness.

[c] CV = 70% for seedlings.

[d] Seedlings counted within 1-m radius of target plant.

[e] Tukey's HSD used to calculate means separations. Values within a column with same letters are not different ($P \leq 0.05$).

[f] Not significantly different than 0 ($P \leq 0.05$) according to t-test performed on least squares mean.

Table 6: Effect of glyphosate and imazapic applied in September 2011 on buffelgrass tiller number and greenness at the Pusch Ridge site. Data are least squares means for data collected 24 MAT.

Glyphosate rate	Imazapic rate	LS mean tillers[a]	LS mean greenness[b]
kg ae/ha	kg ae/ha	no. tillers/plant	%
0	0	42.1 a[c]	66.7 a
0	0.21	22.0 b	32.5 b
0	0.42	6.4 c	10.9 c
2.52	0	0 d[d]	0.1 d[d]
2.52	0.21	3.7 cd[d]	6.0 cd
2.52	0.42	0 d[d]	0.1 d[d]
3.36	0	0 d[d]	0.1 d[d]
3.36	0.21	0 d[d]	0.1 d[d]
3.36	0.42	0 d[d]	0.1 d[d]
4.20	0	0 d[d]	0.1 d[d]
4.20	0.21	0 d[d]	0.1 d[d]
4.20	0.42	3.3 cd[d]	6.0 cd
5.04	0	0 d[d]	0.1 d[d]
5.04	0.21	0 d[d]	0.1 d[d]
5.04	0.42	0 d[d]	0.1 d[d]

[a] CV = 60% for tillers.

[b] CV = 80% for greenness.

[c] Tukey's HSD used to calculate means separations. Values within a column with same letters are not different ($P \leq 0.05$).

[d] Not significantly different than 0 ($P \leq 0.05$) according to t-test performed on least squares mean.

Table 7: Effect of glyphosate and clethodim applied in July 2012 on buffelgrass tiller number and greenness at the Pusch Ridge site. Data are least squares means for data collected 12 MAT.

Glyphosate rate	Clethodim rate	LS mean tillers[a]	LS mean greenness[a]
kg ae/ha	kg ae/ha	no. tillers/plant	%
0	0	35.6 a[b]	56.0 a
0	0.28	27.3 a	50.0 a
0	0.56	29.7 a	48.0 a
0.63	0	10.3 bc	15.1 bc
0.63	0.28	8.3 bcd	15.1 bc
0.63	0.56	11.0 b	22.1 b
1.25	0	0.3 ed[c]	2.2 cd[c]
1.25	0.28	0.1 ed[c]	1.2 d[c]
1.25	0.56	1.2 cde[c]	2.2 cd[c]

[a] CV = 40% for both tillers and greenness.

[b] Tukey's HSD used to calculate means separations. Values within a column with same letters are not different ($P \leq 0.05$).

[c] Not significantly different than 0 ($P \leq 0.05$) according to t-test performed on least squares mean.

Table 8: Effect of glyphosate and clethodim applied in August 2011 on buffelgrass tiller number and greenness at the Robles Junction site. Data are least squares means for data collected 24 MAT.

Glyphosate rate	Clethodim rate	LS mean tillers[a]	LS mean greenness[a]
kg ae/ha	kg ae/ha	no. tillers/plant	%
0	0	61.1 a[b]	62.5 a
0	0.18	34.9 ab	43.8 ab
0	0.28	30.9 ab	43.8 ab
0.63	0	20.9 b	26.4 ab
0.63	0.18	25.0 ab	32.6 ab
0.63	0.28	24.9 ab	38.8 ab
0.84	0	27.4 ab	33.9 ab
0.84	0.18	12.8 b[c]	23.9 b
0.84	0.28	15.3 b[c]	17.7 b

[a] CV = 50% for both tillers and greenness.

[b] Tukey's HSD used to calculate means separations. Values within a column with same letters are not different ($P \leq 0.05$).

[c] Not significantly different than 0 ($P \leq 0.05$) according to t-test performed on least squares mean.

Table 9: Effect of glyphosate and clethodim applied in September 2012 on buffelgrass tiller number and greenness at the Robles Junction site (B3). Data are least squares means for data collected 12 MAT.

Glyphosate rate	Clethodim rate	LS mean tillers[a]	LS mean greenness[b]
kg ae/ha	kg ae/ha	no. tillers/plant	%
0	0	33.0 a[c]	50.0 a
0	0.28	34.4 a	65.0 a
0	0.56	25.3 ab	60.0 a
0.63	0	6.1 c[d]	10.2 b[d]
0.63	0.28	5.8 bc[d]	7.7 b[d]
0.63	0.56	8.1 bc[d]	14.0 b[d]
1.25	0	0.6 c[d]	1.6 b[d]
1.25	0.28	7.4 c[d]	9.1 b[d]
1.25	0.56	0.6 c[d]	1.6 b[d]

[a] CV = 70% for tillers.

[b] CV = 60% for greenness.

[c] Tukey's HSD used to calculate means separations. Values within a column with same letters are not different ($P \leq 0.05$).

[d] Not significantly different than 0 ($P \leq 0.05$) according to t-test performed on least squares mean.

Table 10: Effect of imazapyr applied in July 2012 and February 2013 on buffelgrass tiller number and greenness at the Pusch Ridge site (A5). Data are least squares means for data collected 6 and 12 MAT.

Imazapyr rate	Season	LS mean tillers[a]	LS mean greenness[a]
kg ae/ha		no. tillers/plant	%
0	summer	40.0 a[b]	56.0 a
0	winter	31.7 a	58.0 a
0.56	summer	9.8 b	19.1 b
0.56	winter	5.8 bc[c]	11.2 bc
1.12	summer	4.4 bc[c]	9.2 bc
1.12	winter	0 c[c]	0.3 c[c]
1.68	summer	1.4 c[c]	2.3 c[c]
1.68	winter	0 c[c]	0.3 c[c]

[a] CV = 50% for both tillers and greenness.

[b] Tukey's HSD used to calculate means separations. Values within a column with same letters are not different ($P \leq 0.05$).

[c] Not significantly different than 0 ($P \leq 0.05$) according to t-test performed on least squares mean.

Table 11: Effect of imazapyr applied in September 2012 and March 2013 on buffelgrass tiller counts per plant, visually estimated plant greenness, and seedling counts at the Robles Junction site (B5). Data are least squares means for data collected 6 and 12 MAT.

Imazapyr rate	Season	LS mean tillers[a]	LS mean greenness[a]	LS mean seedlings[b]
kg ae/ha		no. tillers/plant	%	no. seedlings[c]
0	summer	26.0 a	47.5 a[c]	21.0 a
0	winter	23.5 a	38 ab	16.6 a
0.56	summer	13.4 a	28 ab	1.4 b[d]
0.56	winter	19.3 a	25 ab	0.6 b[d]
1.12	summer	10.9 a	11 b[d]	0.5 b[d]
1.12	winter	18.1 a	18 ab	0.1 b[d]

[a] CV = 50% for both tillers and greenness.

[b] CV = 70% for seedlings.

[c] Tukey's HSD used to calculate means separations. Values within a column with same letters are not different ($P \leq 0.05$).

[d] Not significantly different than 0 ($P \leq 0.05$) according to t-test performed on least squares mean.

Figures

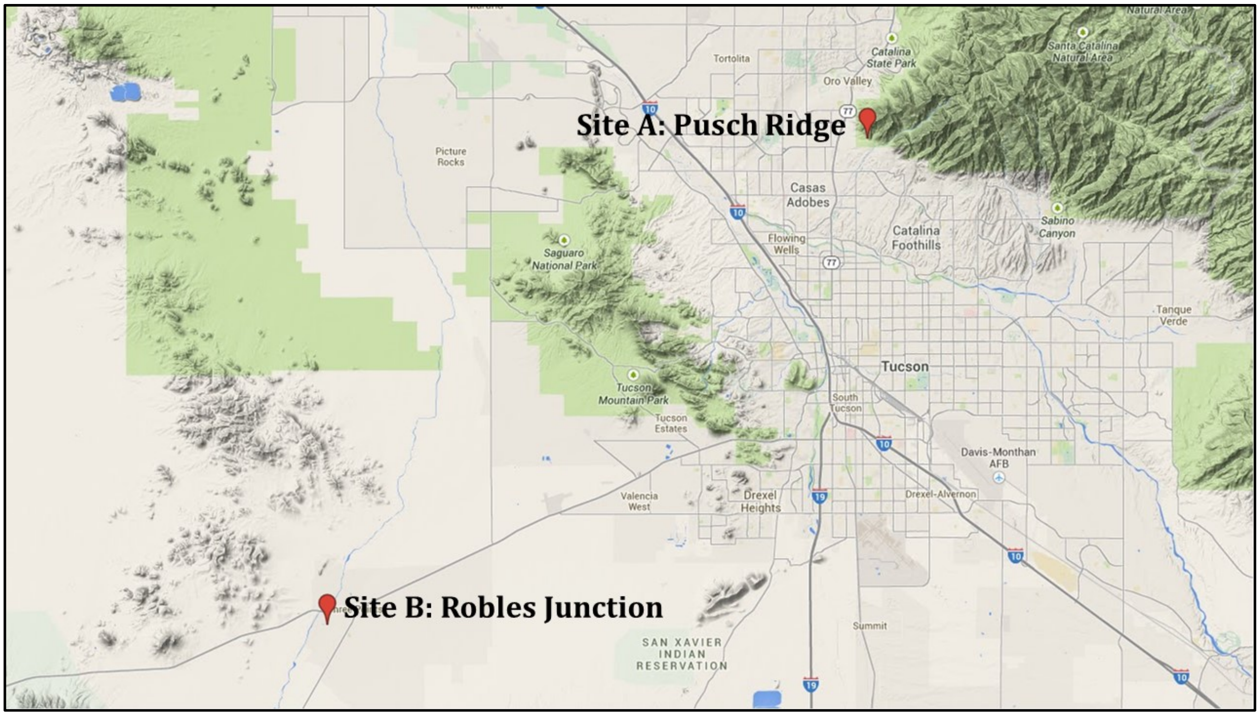

Figure 1: Relative location of study site A (Pusch Ridge) and study site B (Robles Junction).

Figure 2: View of Site A (32.357407, -110.952955), located at the western edge of the Santa Catalina Mountains on a southwest-facing slope near Pusch Ridge on the northern edge of metropolitan Tucson, AZ.

Figure 3: View of Site B (32.068040, -111.333580), located in the Altar Valley in a former agricultural field 43 km west of Tucson near the town of Robles Junction, A

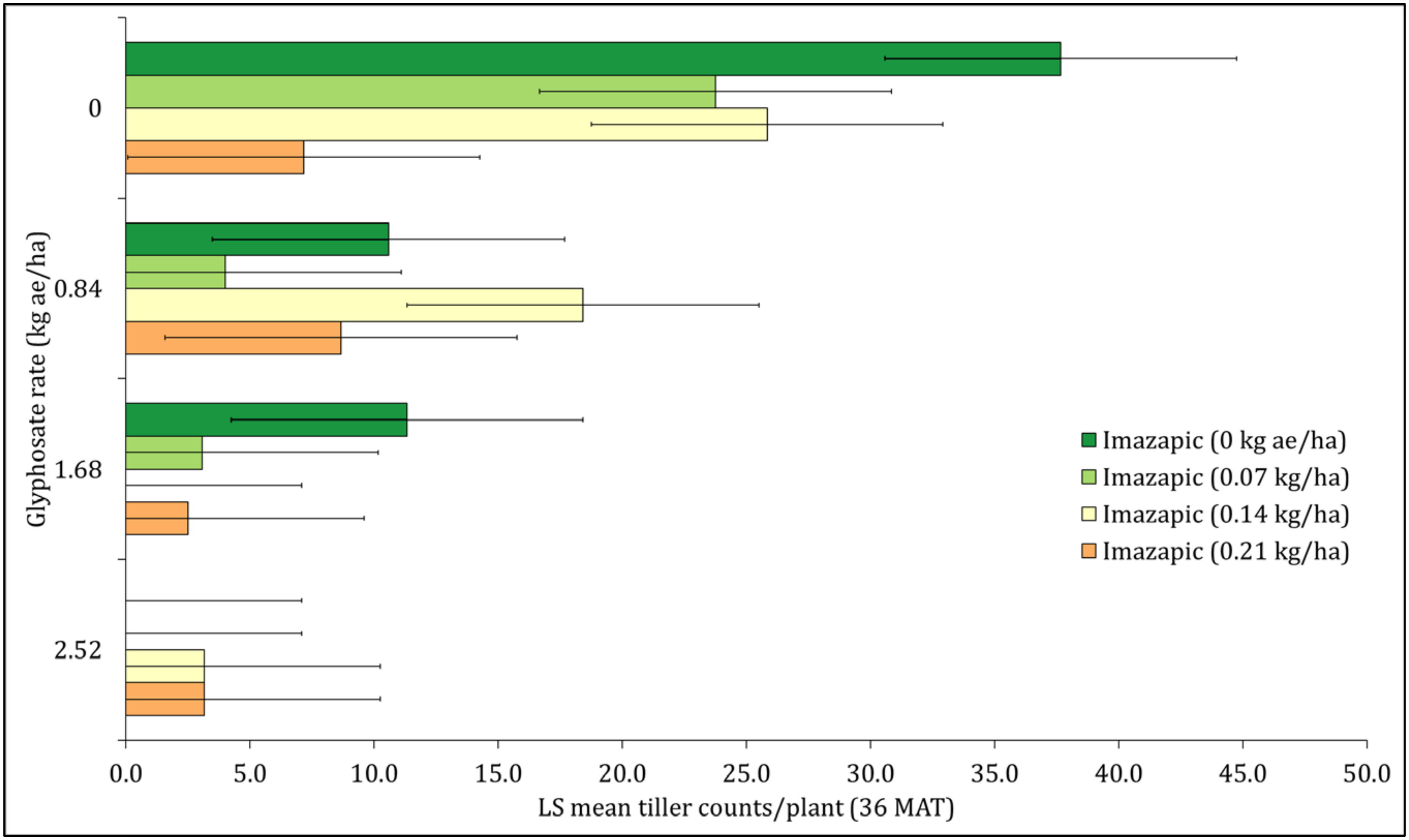

Figure 4: Effects of different tank mixtures of glyphosate and imazapic applied in August 2010 on buffelgrass least square mean tiller number at the Pusch Ridge site, collected 36 months after treatment (MAT). Error bars are 95% confidence

intervals.

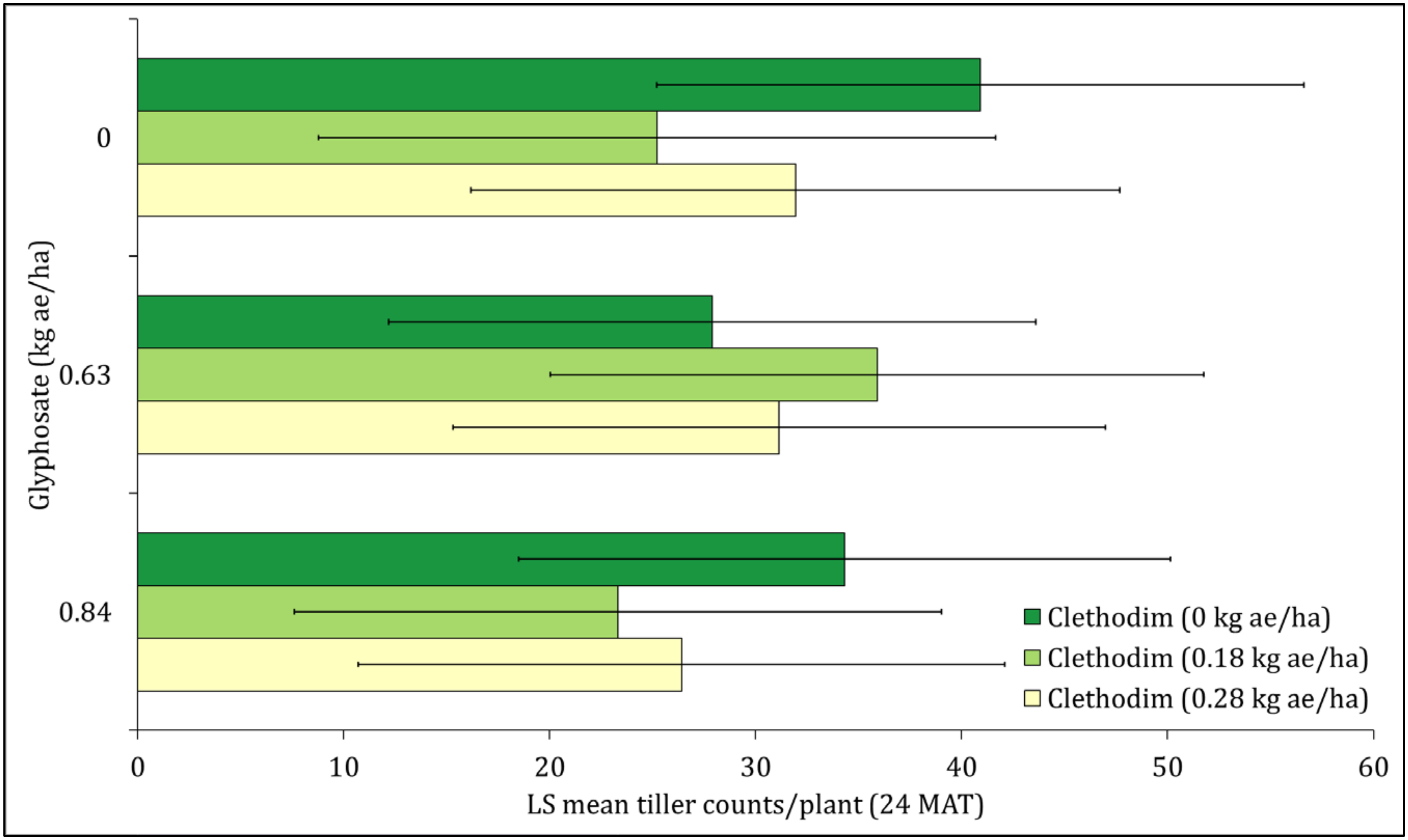

Figure 5: Effects of different tank mixtures of glyphosate and clethodim applied in August 2011 on buffelgrass least square mean tiller number at the Robles Junction site, collected 24 months after treatment (MAT). Error bars are 95% confidence

intervals.

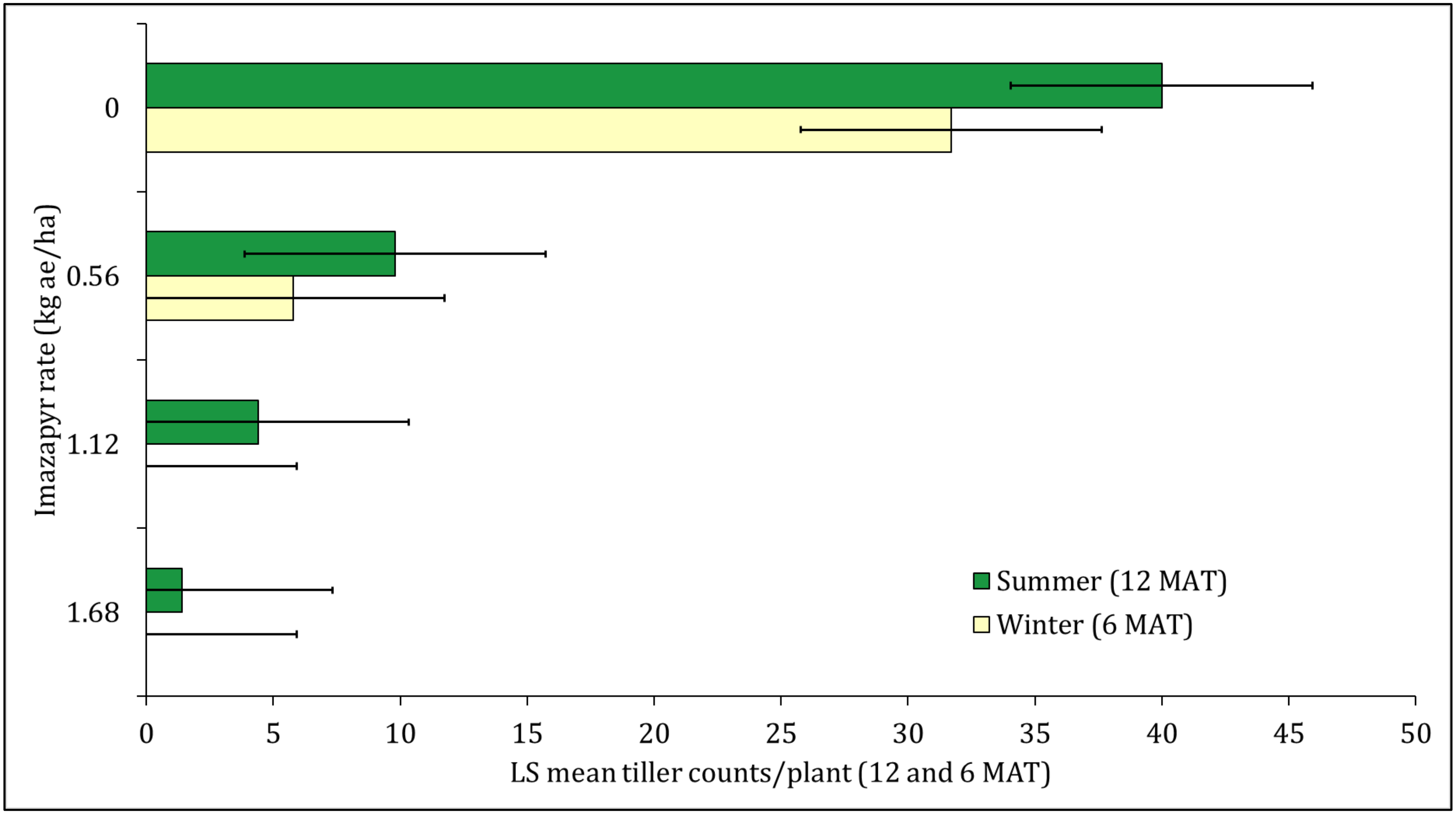

Figure 6: Effects of summer (active growth) and winter (dormant) applications of imazapyr on buffelgrass least square mean tiller number at the Pusch Ridge site, collected 12 (summer) and 6 (winter) months after treatment (MAT). Error bars are 95% confidence intervals

APPENDIX C: Response of buffelgrass (*Cenchrus ciliaris*) and non-target native vegetation to aerial application of glyphosate

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Abstract

Rapid spread of buffelgrass in the Sonoran Desert is outpacing ground-based management efforts in many situations. Further, many buffelgrass populations are located on steep slopes in backcountry areas that are essentially inaccessible on foot. To overcome these limitations, federal agencies and two local jurisdictions conducted a field trial that applied glyphosate by air in the southern Tucson Mountains southwest of Tucson, AZ in the summer of 2010. We subsequently investigated the efficacy of the helicopter broadcast applied glyphosate for buffelgrass control and the concomitant injury these applications caused to native non-target vegetation. Two rates of glyphosate, 1.13 and 2.23 kg acid equivalent ha^{-1}, were each sprayed in carrier volumes of 46.9 and 93.8 L ha^{-1} of water for a total of four treatments. However, a related study (Thistle et al., 2014) found that the rates of glyphosate deposited on the ground were much less than what was released from the

helicopter (26-76% of released) due to the height of spray release, droplet size and environmental factors. Sites were chosen to contain both buffelgrass, which was monitored for plant health one-year following glyphosate application, and representative non-target vegetation; over 1,600 individual non-target plants were marked and monitored for at least two years after the glyphosate applications. Buffelgrass was also monitored under paloverdes above 1.5 m in height and in plant interspaces to evaluate canopy effects. Glyphosate deposition decreased with increasing time of day, which was likely due to atmostpheric instability (Thistle et al. 2014). These times also coincided with all 46.9 L ha^{-1} carrier rate treatments, potentially confounding carrier rate as a model factor. Buffelgrass control increased with increasing glyphosate deposition rate and glyphosate deposition rate had a significant interaction with plant location in relation to paloverde canopies. Saguaros and other cacti were not affected by any of the treatments in year one or two post glyphosate application. Generally, response of non-target vegetation followed a trend where shrubs of increasing height incurred less damage (measured as change in plant greenness) from herbicide treatments than did smaller life forms. Brittlebush, limberbush, ocotillo, little leaf paloverde, triangle leaf bursage, and wolfberry were the only non-target plants in the study to experience damage from any of the treatments. Buffelgrass had a higher rate of increasing damage per kg ae ha^{-1} glyphosate deposition than non-target vegetation except limberbush. Buffelgrass injury was consistent with ground-based applications when deposited rate of glyphosate is considered. Although damaged, buffelgrass was not killed at rates applied in this study. The protocols used in this study need to be modified to kill buffelgrass plants in a single application. Although raising glyphosate rates may be required, as a first step helicopter applications should be made early in the morning before thermal updrafts on steep slopes disperse spray droplets. Larger spray droplets and anti-evaporant adjuvants may also help more glyphosate reach the ground. This may result in greater injury or possible mortality of certain non-target species if present in buffelgrass stands. Finally, non-target damage can be reduced by limiting aerial application of glyphosate to higher-density buffelgrass stands where susceptible non-target species are no longer present.

Nomenclature: Glyphosate; buffelgrass, *Cenchrus ciliaris* L.; fairy duster, *Calliandra eriophylla* Benth.; limberbush, *Jatropha cardiophylla* (Torr.) Müll. Arg.; ocotillo, *Fouquieria splendens* Engelm.;triangleleaf bursage, *Ambrosia deltoidea* (Torr.) W.W. Payne; wolfberry, *Lycium* spp. L.

Key words: Aerial application, C_4 perennial bunchgrass, deposition, non-target effects.

Introduction

Buffelgrass (*Cenchrus ciliaris* L.) is a perennial African bunchgrass that aggressively outcompetes native Sonoran Desert species for water (Eilts and Huxman 2013) and may initiate a grass-fire cycle resulting in ecosystem transformation (Butler and Fairfax 2003; D'Antonio and Vitousek 1992; McDonald and McPherson 2011; McDonald and McPherson 2013; Miller et al. 2010). Infestations often occur in dense stands on steep rocky slopes that can be practically inaccessible from the ground or pose a safety hazard to field crews (T. Bean, personal observation). Ground-based buffelgrass control efforts include manual removal using digging bars and picks, and glyphosate application. Glyphosate application is much less resource intensive on a per-area basis compared to manual removal, allowing much more area to be treated in any given year (D. Backer, USDI National Park Service, personal communication), which is very important considering the exponential spread rates of buffelgrass in some areas of the Sonoran Desert (Olsson et al. 2012b). Glyphosate is the most commonly used herbicide for controlling buffelgrass in this region (Backer and Foster 2007). Glyphosate requires foliar uptake by the target species and is optimally effective during what are often short and unpredictable time frames occurring following the onset of summer rains in July or August (Bean et al. 2014). This is when buffelgrass is actively growing, allowing for herbicide uptake and translocation to meristematic tissues within the plants (Devine et al. 1993; Ross and Lembi 2008). As of 2009, over 4,000 ha (10,000 ac) of buffelgrass existed in southern Arizona on federal land alone and at most, 100’s of ha are being treated on an annual basis (D. Backer, USDI National Park Service; S. Biedenbender, USDA Forest Service; D. Tersey, USDI Bureau of Land Management; personal communications). In other words, although ground-based glyphosate application is capable of treating much more area per unit time than manual control, neither can keep pace with buffelgrass spread.

To address the challenge of difficult access and constraints on managers' ability to treat enough area in a given year to keep pace with spread rates, local and national land managers joined together to evaluate the use of helicopters to broadcast spray glyphosate for buffelgrass control (Backer et al. 2012). Aerial herbicide application is routinely used for weed control in agriculture and forestry, with such applications being most successful when weed infestations are homogenous and large in area with uniform terrain (Payne 1993; Reddy et al. 2010; Riley et al. 1991; Yates et al. 1978; Zutter et al. 1988). However, buffelgrass infestations in the Sonoran Desert offer several unique challenges, including steep, rugged terrain, relatively small target areas, extreme heat, and the presence of native plants unique to this landscape, particularly saguaro ([Engelm.] Britton & Rose), which can reach over 15 m (50 ft) in height and thus also pose a safety threat to the helicopter operator and require that herbicide must be applied from an unusually high altitude (Thistle et al. 2014). Three federal agencies, two local jurisdictions and University of Arizona scientists collaborated to design this study (Backer et al. 2012) starting in 2008, culminating in a field trial that was conducted in summer 2010 and monitored through 2013 with the goal of investigating some of the engineering and biological questions regarding the implementation and feasibility of aerially applying herbicides to manage an invasive C_4 perennial bunchgrass in this environment. The engineering aspects, including what was learned regarding application technique and the operational approach to spraying under such conditions, as well as detailed documentation of local weather data at the time of glyphosate application and data on herbicide deposition and drift were comprehensively presented by (Thistle et al. 2014). This case study discusses the biological efficacy of these applications in controlling buffelgrass and the effects on non-target species. Study sites were located in areas of low buffelgrass density on the landscape (<1 plant m^{-2} [0.1 plant ft^{-2}], although individual patches might have 2 to 4 plants m^{-2} [0.2 to 0.4 plants ft^{-2}]) so that native non-target vegetation could be monitored for glyphosate injury. Thus the sites may not necessarily be representative in terms of species composition of areas where herbicides may actually be applied by air. Buffelgrass and non-target vegetation were monitored for glyphosate damage in paired treated and untreated areas for up to three years following glyphosate application depending on species and plant size. Findings in the Thistle et al. (2014) study were critical to interpreting biological results, as glyphosate deposition rates were found to differ greatly from what was previously thought to have been applied.

Although resources did not allow a more comprehensive set of trials to be conducted, this pilot project offers a starting point for the design of more thorough aerial application trials and improved monitoring for large scale aerial applications in the field. This project also provided essential data on the feasibility of aerial glyphosate applications for buffelgrass control and the concomitant environmental impacts to assist land managers in completing legal documentation such as an Environmental Assessment or Environmental Impact Statement. Specifically, our goal was to use the available monitoring data to answer several questions of particular relevance to buffelgrass management. First, can buffelgrass be controlled by broadcast spraying glyphosate with a helicopter? Can buffelgrass under tree canopies be controlled using this method? Which glyphosate rates provide the greatest control and how does control compare to ground-based applications? Do these glyphosate rates injure native non-target species and, are there patterns of susceptibility in non-target plant species or life forms? What can be changed for better control in future helicopter glyphosate applications or better monitoring of glyphosate effects on buffelgrass and non-target plant species?

Materials and Methods

Study sites. Our study sites were located in the Arizona Upland Subdivision of the Sonoran Desert, where the overstory is dominated by little leaf paloverde (*Parkinsonia microphylla* Torr.) and saguaro (*Carnegiea gigantea* [Engelm.] Britton & Rose). The study sites were located in the southern part of the Tucson Mountains, west of Tucson, AZ, on properties owned by Pima County, the City of Tucson, or managed by the Bureau of Land Management (Figure 1). The study sites were at approximately 700 to 1,000 m (2,300 to 3,300 ft) in elevation on well-drained, shallow (3 to 5 cm or 1 to 2 in), very to extremely gravelly sandy loam and fine sandy loam soils on slopes of 30 to 50% (Cochran and Richardson 2003). Long-term mean precipitation (1981-2010, data from the Tucson International Airport) is 280 mm (11 in) annually of which 179 mm (7 in) is received during the summer (June through October).

Experimental design. We applied five herbicide treatments including a control and a full-factorial combination of two rates of glyphosate and two water carrier volumes (Table 1, this paper). Applied glyphosate rates were 1.13 and 2.23 kg ae (acid equivalent) ha^{-1} (1.01 and 1.99 lb ae ac^{-1}) and carrier volumes were 46.9 and 93.8 L ha^{-1} (5 and 10 gal ac^{-1}) water.

However, actual deposition of glyphosate differed greatly from these rates (Thistle et al. 2014). In particular, applications later in the morning were most likely subjected to atmospheric instability and thermal updrafts on steep slopes that evaporated and dispersed spray droplets far from the vicinity of the plots resulting in tremendous loss of herbicide (Table 1, this paper). Further, all 46.9 L ha^{-1} carrier volume treatments occurred during the period of atmospheric instability, confounding carrier volume as an effect. There was a 2.5 hour delay, a change of 6 °C and 20% relative humidity before the second set of applications at the lower carrier volume that seriously comprised the goals of this study as judged from the deposition data (Thistle et al. 2014). In this study we will focus on the response of buffelgrass and non-target vegetation to deposited and not applied glyphosate rates. From the Materials and Methods section and Table 1 in the Thistle et al. (2014) paper, we can infer that there were two flights with the first applying a carrier volume of 93.8 L ha^{-1} and a second applying a carrier volume of 46.9 L ha^{-1}. Within each flight two rates of glyphosate were applied. Hereafter these are referred to as Flight 1A, 1B, 2A, and 2B (Table 1, this paper). Glyphosate deposition was calculated as 0.86, 1.65, 0.29, and 0.76 kg ae ha^{-1}, for flights 1A, 1B, 2A, and 2B, respectively. Detailed information and discussion on the spray application and herbicide deposition can be found in Thistle et al. (2014).

Herbicide treatments were applied on the morning of 19 August 2010. Subsequent monitoring efforts were conducted when plants were green and actively growing following the onset of summer rains, which typically begin in July. Each treatment transect was paired with a nearby untreated control transect. Paired control and treatment transects (45 to 50 m [150 to 160 ft] each) were located parallel to each other, with the treatment transect bisecting a 0.3 to 47 ha (0.7 to 1.5 ac) treated area; control transects were located at least 10 m (30 ft) outside the treated area to avoid overspray. Some minor overspray did occur on control transects (Thistle et al. 2014). A two-meter wide belt transect was placed along each of the control and treatment transects in which buffelgrass was monitored. Monitored buffelgrass plants were at least 0.3 m (1 ft) wide and tall to avoid selecting seedlings and immature (first-year) plants that are more susceptible to glyphosate (Devine et al. 1993; Ross and Lembi 2008). For each of the five life form classes, metal tags were attached to at least 10 individuals of native non-target species so that their individual response in each treatment and control transect pair could be monitored over time. Perennial grasses other than buffelgrass not usually a dominant component of the native plant community, and were not monitored, although it was assumed that damage incurred by buffelgrass would be approximately representative for other C_4 grasses that may occur in the area. Similarly, annual grasses and forbs were not monitored due to their unpredictable emergence on an annual basis, although it was assumed that they would be susceptible to glyphosate if they were sprayed. Also, annuals will reemerge from the soil seed bank since there are no residual effects from glyphosate application. A total of 12 plots (3 for each treatment) were randomly located to the extent possible. We selected plots based on several factors that included representative vegetation community composition that would allow for an assessment of non-target effects, terrain, slopes and soils typically infested by buffelgrass, sufficient existing stands of buffelgrass, access for researchers and monitoring crews, and proximity to a staging area for the helicopter and associated equipment and supplies.

We analyzed the six week, one-year, two-year, and three-year post-treatment greenness data by life form and also by species when there were enough of a particular species present (at least 25 individuals). One-year and two-year-old buffelgrass plants were indistinguishable in the field, therefore buffelgrass was excluded from the two-year data. Three-year monitoring was only performed for the largest and longest-lived life forms (saguaro, other cacti and trees). We have found that buffelgrass plants and native species often require one year or more for herbicide effects to be adequately evaluated (Bean and McCloskey 2014). Thus, the six-week data are not presented because they superseded by longer term data. Similarly, three-year data (data not shown) are not presented because species most likely to incur damage (smaller, less woody plants and non-cacti species) were not monitored. Further, the third year of monitoring took place in 2013, which was a very dry year for summer moisture, with the Tucson Airport receiving only 53% of the long-term (1981-2010) seasonal (July to October) average. This likely suppressed plant growth, making it difficult to distinguish treatment responses from controls. Our response variable was visually estimated percent greenness of the plants from a single observer, though consensus among multiple observers was obtained when classification was difficult. Visually estimated greenness of non-target species were grouped into four classes of 0 to 10%, 10 to 40%, 40 to 75%, and 75 to 100% green. Buffelgrass estimated greenness was grouped into three classes of 0 to 10%, 10 to 75%, and 75 to 100% green (Bean et al. 2014). All plants monitored were verified to be in the highest greenness class in the week prior to glyphosate application, therefore relative to greenness, this is not a random sample. Midpoints of greenness classes were used for statistical analysis. To meet assumptions of normality, analysis was conducted using arcsine-transformed values for the percent green values (Gomez and Gomez 1984). All treatment means were back-transformed for purposes of reporting.

The original study design was to use glyphosate application rate and carrier volume as model effects, but as noted previously, glyphosate deposition rates varied widely from application rates and carrier volumes were confounded by atmospheric conditions. Because different helicopter flights corresponded to different glyphosate deposition rates, flight numbers were instead used as model effects in lieu of applied glyphosate rates and carrier volumes. We assumed a completely randomized but unbalanced design and considered individual plots, flight numbers (treatments), and their interaction to be the treatment factors (fixed effects, all treated as class variables in this analysis). Although three plots were allocated per treatments, they were restricted to areas with buffelgrass infestations and therefore were located in across a wide space with different soil, topography and vegetation characteristics and were not considered true replicates or random effects. We used the PROC GLM procedure in SAS (SAS 2012, Version 9.3; SAS Institute, Inc., Cary, NC) to determine if differences existed in visually estimated plant greenness (as a proxy for glyphosate damage) among glyphosate treatments (control and four glyphosate rates) for buffelgrass, non-target vegetation life forms, and selected non-target species. Mean separations were performed using the Duncan's Multiple Range Test with $P \leq 0.05$. For buffelgrass alone, we also used the PROC GLM procedure to determine if differences existed in visually estimated plant greenness among glyphosate treatments (control and four glyphosate rates) by paloverde canopy (under canopy or in the open). We then performed simple linear regression in PROC REG in SAS treating glyphosate deposition rate as a continuous variable. Confidence intervals ($P \leq 0.05$) of regression coefficients were used to evaluate buffelgrass and non-target vegetation response to increasing glyphosate deposition rates.

For non-target species, we performed separate analysis for each life form and species (Table 2). Non-target species were restricted to perennials and life forms were categorized by succulence or height, including saguaro, other cacti, subshrubs (<0.47 m [1.6 ft]), shrubs (0.47 to 1.5 m [1.6 to 4.9 ft]), trees (>1.5 m [4.9 ft]). For those non-target species that were present in multiple life forms, we ran a full generalized linear effects model (all possible interactions) including life form as a treatment. Only those species with a total occurrence of at least 25 individuals on which monitoring was performed were listed in the analysis report. None of the species were abundant enough to analyze the effect of height (life form) on herbicide susceptibility for individual species (data not shown). We monitored buffelgrass plants that were located inside the drip line of little leaf paloverde tree canopies as well as buffelgrass plants not under canopies, and analyzed them separately.

Results and Discussion

Buffelgrass. We found a significant interaction (F=15.48, $P \leq 0.0001$) between helicopter flight (treatment) and canopy (under paloverde canopy or in the open), and concluded that buffelgrass responded differently to treatments depending on the location of the plant in relation to tree canopies. No deposition measurements were taken under paloverde canopies, so a comparison of the simple linear regression results for buffelgrass under canopies Vs. buffelgrass in the open is not appropriate. Instead, we must rely on comparisons among individual flights and the control. At one year post glyphosate application, buffelgrass plants not under little leaf paloverde canopies that were sprayed with flight 1B (1.65 kg ae ha^{-1} glyphosate) were significantly less green (15.8%) than those in all other treatments and the control (75.8%) (Table 3). Buffelgrass plants under little leaf paloverde canopies that were sprayed with flight 2B (0.76 kg ae ha^{-1} glyphosate) were significantly less green (13.6%) than those in all other treatments and the control (76.9%) (Table 3). The difference between these two flights was that 2B had less carrier volume, either because of evaporation and dispersal of droplets due to unstable atmospheric conditions or because the volume was mixed lower to start. Thus, it's possible that even though 2B exhibited less glyphosate deposition than 1B, the glyphosate concentration of droplets deposited in 2B may have been higher. Regardless, at 15.8 and 13.6% green, complete buffelgrass mortality was not achieved for either of the most successful treatments, though it appears that buffelgrass under paloverde canopies had a similar response to buffelgrass plants in the open, even though glyphosate rates for plants in the open were much higher. Buffelgrass plants in the open had a significant regression of greenness based on glyphosate rate, suggesting that for every kg ae ha^{-1} of glyphosate deposited greenness decreases 42.1% (Table 5). Because buffelgrass was not monitored in year two it is unknown whether if damaged plants finally died or instead recovered.

For comparison, we found in a separate study that 2.52 kg ae ha^{-1} (2.25 lbs ae ac^{-1}) of glyphosate in a carrier volume of 190 L ha^{-1} (20 gal ac^{-1}) was sufficient to consistently kill mature buffelgrass plants in a single application when applied from the ground (Bean and McCloskey 2014). We know from Thistle et al. (2014) that the herbicide and water mixture was evaporating or drifting off-target before reaching the ground, resulting in a lower effective application rate. The buffelgrass greenness response to glyphosate observed in this study at the 1.65 and 0.76 kg ae ha^{-1} rates were similar to that observed in the Bean and McCloskey (2014) ground-based study at rates of 1.68 and 0.84 kg ae ha^{-1} (1.50 and 0.75 lbs ae ac^{-1}). To ensure that more glyphosate is deposited on the ground, helicopter applications should occur in the early morning when temperatures are cooler and relative humidity is higher. According to the Thistle et al. (2014) paper, relative humidity was approximately 60% during the time when the high carrier volume was applied and fell to 38% later in the day when the lower carrier volume was applied. While this does make commenting on the effects of carrier volume difficult, it does strongly suggest that more glyphosate will reach the ground if applied as early as possible following dawn, likely resulting in higher buffelgrass mortality.

Life forms. In the ANVOA analysis, neither saguaro nor other cacti greenness was significantly different among treatments or the control at one or two years following application (Tables 3 and 4). Glyphosate is known to be ineffective in controlling *Opuntia* spp. (Felker and Russell 1988; Monteiro et al. 2005), and it is possible that other cacti species are also tolerant to glyphosate as herbicides documented to provide effective control of cacti such as MSMA (monosodium methanearsonate), triclopyr, imazapyr, 2,4-D and picloram (Malan and Zimmerman 1988; Petersen et al. 1988) have different modes of action than glyphosate (Senseman 2007). Our results also indicate that saguaro and other cacti do not appear to be susceptible to a single application of glyphosate, at least at the herbicide rates and carrier volumes applied in this study. This is important because it has been noted that saguaro, which are a very high-value component of this vegetation, are often the only remaining species in buffelgrass stands more than 10-15 years after invasion (Olsson et al. 2012a). Therefore the potential for non-target damage in general, and specifically for damage to the iconic saguaro, may be minimal for older buffelgrass stands. Repeated glyphosate applications in a single season or consecutive years may injure cacti, though this is unlikely to occur if an aerial application provides substantial buffelgrass kill and follow up treatments are made by ground.

In years one and two, tree and shrub greenness was significantly less than the control with the flight 2B application (Tables 3 and 4). A possible explanation is that the sparse canopy of tree and shrub species provided less leaf surface area for herbicide interception and uptake and plants were more susceptible to the higher glyphosate concentration per volume of water in this treatment. Trees and shrubs increased from 66.8 and 57.2% green in year one to 72.7 and 64.9% in year two with the flight 2B application (Tables 3 and 4), suggesting they may recover or at least remain stable over the longer term without repeated applications. In year one, trees had a significant regression of greennesss on glyphosate deposition rate, suggesting that for each kg ae ha^{-1} of glyphosate, tree greenness will be reduced by 3.5% (Table 5). In year two the regression coefficient was not significant, supporting the previously stated assertion that tree damage may be short-lived and will recover within a year following glyphosate application.

In both years one and two, subshrub greenness was significantly reduced compared to the control with both flights 1B and 2B (Tables 3 and 4). No increase in greenness was noticeable in year two, suggesting that subshrub growth may take longer to recover than trees and shrubs or may experience reduced growth in the long term for flights 1B and 2B. Curiously, flight 1A resulted in higher glyphosate deposition (0.86 kg ae ha^{-1}) than flight 2B, but flight 1A buffelgrass greenness was not significantly different that the control. This supports the concept that the lower applied carrier rate in flight 2B may have resulted in higher glyphosate concentrations per volume of water deposited. In both years, subshrubs had significant regressions of greenness based on glyphosate rate, suggesting that for every kg ae ha^{-1} of glyphosate deposited greenness decreases 9.9 and 14.1% (Tables 5 and 6). This supports the idea that subshrub recovery from glyphosate application may take longer than for the larger lifeforms of trees and shrubs.

Although similarities in greenness response to glyphosate application do exist among life forms or plants of the same height, these are artificial groupings and caution should be made in generalizing these results. A more prudent approach might have been to consider individual species when accounting for differences in juveniles and mature plants, which have different susceptibilities to herbicide application (Devine et al. 1993; Ross and Lembi 2008). Generalizations would be more robust if different species were grouped by morphological and physiological similarities (as for cacti), and not simply height. Plant age and stage of growth, height, leaf orientation, leaf and stem surface characteristics, differences in metabolic pathways, etc. are just a few very important determinants of herbicide susceptibility (Ross and Lembi 2008).

Species. For the ANOVA analysis of species-specific effects, greenness did not decline among treatments or the control in year one or two for barrel cactus (*Ferocactus wislizeni* [Engelm.] Britton & Rose), fairy duster (*Calliandra eriophylla* Benth.), jojoba (*Simmondsia chinensis* [Link] C.K. Schneid.), mallow (*Abutilon* spp. Mill.), slender janusia (*Janusia gracilis* A. Gray), staghorn cholla (*Cylindropuntia versicolor* [Engelm. ex J.M. Coult.] F.M. Knuth), whitethorn acacia (*Vachellia constricta* [Benth.] Seigler & Ebinger), white ratany (*Krameria grayii* Rose & Painter), and saguaro (Tables 3 and 4). Unlike the other species mentioned above, whitethorn acacia had a significant regression coefficient in year two for decline in greenness by glyphosate deposition rate, suggesting that for every kg ae ha^{-1} of glyphosate deposited greenness decreased 17.5% (Table 6). The remainder of these species appear to be tolerant to a single applications with glyphosate deposition rates documented in this experiment. Most of the affected species showed highest sensitivity to flight 2B, which likely had a lower carrier rate but a but also had a lower glyphosate deposition rate than flight 1A, which none of the species responded to.

The only exception was limberbush (*Jatropha cardiophylla* [Torr.] Müll. Arg.), in which greenness was reduced dramatically compared to the control at both 1.65 and 0.76 kg ae ha^{-1} glyphosate rate regardless of applied carrier volume (Tables 3 and 4). At 5% green, it was impossible to distinguish dead and living plants, and if limberbush does recover at some point in the future, it will likely have to resprout from below-ground meristems or germinate from seed. In both years, limberbush had significant regressions of greenness based on glyphosate rate, suggesting that for every kg ae ha^{-1} of glyphosate deposited greenness decreases 37.9 and 56.2% (Tables 5 and 6). This was the only species with regression coefficients of greenness on glyphosate deposition rate similar to glyphosate (Table 5) and should be considered highly susceptible to the rates of glyphosate applied in this study.

Brittlebush (*Encelia farinosa* A. Gray ex Torr.), ocotillo (*Fouquieria splendens* Engelm.), little leaf paloverde, triangle leaf bursage (*Ambrosia deltoidea* [Torr.] W.W. Payne) and wolfberry (*Lycium* spp. L.) greenness were significantly lower than the control for flight 2B (Tables 3 and 4). Triangle leaf bursage was also susceptible to the glyphosate deposited in flight 2A (0.29 kg ae ha^{-1}). In both years, triangle leaf bursage had significant regressions of greenness based on glyphosate rate, suggesting that for every kg ae ha^{-1} of glyphosate deposited greenness decreases

11.3 and 12.6% (Tables 5 and 6). Both brittlebush and triangle leaf bursage greenness remained relatively stable in years one and two, though brittlebush was quite low at 6.9 and 6.4% in years one and two. Brittlebush did not have a significant regression coefficient of greenness on glyphosate deposition rate, suggesting that some other factor, perhaps applied carrier rate, is more closely related to the reduction in greenness for flight 2B. Little leaf paloverde and wolfberry appeared to increase in greenness in year two, suggesting a capacity to recover from the herbicide rates applied in this study (Tables 3 and 4) . Neither little leaf paloverde nor wolfberry had significant regression coefficients of greenness on deposited glyphosate rate. Ocotillo experienced a decline in greenness from year one (63.0%) to year two (29.0%) (Tables 3 and 4). Ocotillo also had significant regression coefficients for both year one and two, suggesting that for every kg ae ha^{-1} of glyphosate deposited greenness decreases 8.9 and 11.1% (Tables 5 and 6). No differences were found among treatments and the control for the ANOVA analysis of fairy duster, but it did have significant regression coefficients for both years, suggesting that for every kg ae ha^{-1} of glyphosate deposited greenness decreases 8.6 and 16.5%. Other than limberbush, all species with significant regression coefficients did not overlap the 95% confidence interval for buffelgrass' regression coefficient (Table 5).

Of the 34 individual species that were marked for monitoring (Table 1) in this study, only 14 were abundant enough (≥25 individuals) to permit robust statistical analyses (Tables 2 and 3). Of those 14 species, 11 were present in multiple life forms (Table 1), but not in sufficient numbers to test life form (height) as an effect on greenness response to herbicide rates and carrier volumes. This is an artifact of the experimental design, which prioritized selection of non-targets by life form instead of species. A better strategy would have been to pick a smaller number of non-target species that were representative of habitats where aerial glyphosate application might take place, and sample larger numbers of these species, stratifying the sample by plant size or height to capture enough individuals of different developmental stages to allow for estimates of differences in herbicide susceptibility of juvenile vs. mature individuals.

Practical implications and study limitations. This trial demonstrated that applied glyphosate rates varied dramatically from rates actually deposited on the ground. This trial also demonstrated that it was possible to reduce buffelgrass greenness with aerial application with deposition rates of 1.65 (flight 1B) and 0.76 kg ae ha^{-1} (flight 2B) with the lower rate possibly being effective because of a lower applied carrier rate. Flight 1B was more effective at reducing buffelgrass greenness for plants in the open, while flight 2B was more effective at reducing buffelgrass greenness for plants under paloverde tree canopies. Although the buffelgrass was not killed, plants were damaged by this herbicide rate, likely suppressing growth and possibly reproduction, providing some control. However, the given the short time frame available for glyphosate application in a given year (Bean et al. 2014), mortality in a single application is highly desirable for effective management because the possibility of repeat treatments in a single season is unlikely.

However, before considering increasing application rates, several changes should be made to make the helicopter applications more effective so that more glyphosate deposition is achieved. First, it is obvious from the Thistle et al. (2014) study that deposition rates decreased dramatically later in the morning when atmospheric conditions became unstable. There is a need for more work to characterize how much glyphosate reaches the ground. Helicopter applications need to be made after dawn and before mid-morning when thermal updrafts or downdrafts disperse the spray droplets if the helicopter has to operate above saguaro and account for steep slopes. It's also probably appropriate to increase droplet size. Glyphosate rates only need to be raised to compensate for loss of glyphosate. If glyphosate rates are increased, regressions used in Table 5 could be used as a starting point to the appropriate rate and extrapolating. With this estimate of the effective glyphosate application rate, a similar approach could be used to estimate damage to non-target species (Tables 5 and 6) that showed an effect of glyphosate application in this study. As extrapolations beyond the range of existing data, these estimates of the glyphosate application rate needed to kill buffelgrass in a single application and the effect this rate would have on non-target species would not be scientifically robust, though they would provide an informed starting place for future studies or for implementation of aerial spraying, should managers choose to go that route.

Given the data we do have on vegetation response to the treatments, we can offer some suggestions for future studies or implementation of aerial glyphosate application for buffelgrass control. Of the non-target species with enough abundance at the study sites to allow for species-specific estimations of susceptibility, the only species highly likely to experience damage or morality with this approach is limberbush. Other species that were affected by glyphosate, including brittlebush, ocotillo, little leaf paloverde, whitethorn, and wolfberry, are probably more at risk of higher glyphosate deposition rates than species that showed no effect. Similarly, subshrub life forms are probably more at risk of higher glyphosate application rates than life forms that showed no effect. For species not represented in the study, a general rule of thumb for glyphosate susceptibility is that grasses, forbs and smaller shrub species are more susceptible than woody species like larger shrubs and trees or cacti. However, this is probably not relevant, as forbs, grasses, and smaller shrubs are the first species to be displaced by buffelgrass (Olsson et al. 2012a), and so they will not likely be present where aerial application of glyphosate is implemented.

If at all possible, there are several other improvements that should be implemented for future aerial application trials or for monitoring of actual aerial application control efforts that may be implemented. First, each plot had only one transect pair (treatment and control). By establishing multiple transects within each plot, some of the plot-based variation could be accounted for and make it easier to distinguish treatment differences. Second, location of the monitored plants within the plot should be taken into account. Depending on their location within the patch of buffelgrass being sprayed with herbicide from a helicopter, plants are likely to receive different amounts of herbicide. The helicopter sprays along a slope contour, and begins spraying herbicide before it is actually over the patch, and turns off the herbicide before it leaves the patch (T. Bean, personal observation). Thus discrepancies in herbicide deposition are likely to occur based on whether a plant is located on the “approach” end of the path, the middle of the patch, and the “departure” end of the patch. To overcome this, sampling should be stratified by location within the buffelgrass patch. Third, as mentioned previously, life forms were a very artificial grouping, and in the future, specific non-target plant species should be targeted according to abundance in buffelgrass patches or some other relevant prioritization based on management goals or concerns. Each of these species should be sampled across a range of developmental stages to capture the differences in response to glyphosate application between juveniles and mature individuals. Fourth, the response variable measured (greenness) was very coarse in scale, having only four (three for buffelgrass), unevenly spaced classes. We have successfully used a six-category, evenly-spaced class system in previous herbicide response experiments (Bean and McCloskey 2014) and recommend this as a minimum for future trials or monitoring efforts. Also, more quantitative estimates of greenness from handheld NDVI meters or from digital images are highly desirable and can be quickly and easily obtained and batched processed (Bean et al. 2014), providing a compliment or verification to visual estimates. Finally, the Thistle et al. (2014) study indicated that there were discrepancies among herbicide rates applied and the rate of herbicide actually reaching the ground. A possible correction for this would be to use the same tank mixture of herbicide and carrier and apply this with ground-based equipment for comparison purposes.

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Tables

Table 1: Applied glyphosate and water carrier rate, time of application, deposited glyphosate rate, and fraction of deposited V.s. applied glyphosate by treatment (adapted from Thistle et al. 2014).

Treatment	Applied glyphosate[a]	Applied water carrier[b]	Time of application	Deposited glyphosate [a,c]	Fraction of applied[d]
1A	1.13	93.8	07:03	0.86	0.76
1B	2.23	93.8	07:18	1.65	0.74
2A	1.13	46.9	10:17	0.29	0.26
2B	2.23	46.9	10:37	0.76	0.34

[a] kg acid equivalent ha^{-1}.

[b] L ha^{-1}.

[c] Calculated based on deposition of marker dye.

[d] Deposited divided by applied glyphosate.

Table 2: All non-target species monitored in the study, their common names, latin names, and life form classification.

Latin name	Common name	Other cacti[a]	Saguaro[a]	Shrub[a]	Sub-shrub[a]	Tree[a]
Abutilon spp. Mill.	mallow			X	X	
Aloysia wrightii A. Heller	Wright's beebrush			X	X	
Ambrosia deltoidea (Torr.) W.W. Payne	triangleleaf bursage			X	X	
Argythamnia lanceolata (Benth.) Müll. Arg.	narrowleaf silverbush				X	
Brickellia californica (Torr. & A. Gray) A. Gray	brickellbush			X	X	
Calliandra eriophylla Benth.	fairy duster				X	
Carnegiea gigantea (Engelm.) Britton & Rose	saguaro		X			
Celtis pallida Torr.	desert hackberry			X		
Coursetia sp.	babybonnet			X		X
Croton sp. L.	croton				X	
Cylindropuntia bigelovii (Engelm.) F.M. Knuth	teddybear cholla	X				
Cylindropuntia fulgida (Engelm.) F.M. Knuth	chainfruit cholla	X				
Cylindropuntia leptocaulis (DC.) F.M. Knuth	christmas cholla	X				

Latin name	Common name	Other cacti[a]	Saguaro[a]	Shrub[a]	Sub-shrub[a]	Tree[a]
Cylindropuntia versicolor (Engelm. ex J.M. Coult.) F.M. Knuth	staghorn cholla	X				
Encelia farinosa A. Gray ex Torr.	brittlebush			X	X	
Ferocactus wislizeni (Engelm.) Britton & Rose	barrel cactus	X				
Fouquieria splendens Engelm.	ocotillo			X	X	X
Haplophyton crooksii (L.D. Benson) L.D. Benson	cockroachplant				X	
Hibiscus denudatus Benth.	rock hibiscus			X	X	
Janusia gracilis A. Gray	slender janusia			X	X	
Jatropha cardiophylla (Torr.) Müll. Arg.	limberbush			X	X	
Krameria erecta Willd. ex Schult.	littleleaf ratany				X	
Krameria grayii Rose & Painter	white ratany			X	X	
Larrea tridentata (Sessé & Moc. ex DC.) Coville	creosotebush			X		
Lycium spp. L.	wolfberry			X	X	

Latin name	Common name	Other cacti[a]	Saguaro[a]	Shrub[a]	Sub-shrub[a]	Tree[a]
Menodora scabra A. Gray	twinberry				X	
Opuntia engelmannii Salm-Dyck ex Engelm.	prickly pear	X				
Parkinsonia microphylla Torr.	little leaf paloverde			X	X	X
Parthenium incanum Kunth	mariola				X	
Prosopis velutina Wooton	velvet mesquite			X		X
Simmondsia chinensis (Link) C.K. Schneid.	jojoba			X	X	X
Trixis californica Kellogg	trixis			X	X	
Vachellia constricta (Benth.) Seigler & Ebinger	whitethorn acacia			X		X
Senegalia greggii (A. Gray) Britton & Rose	catclaw acacia			X		X

[a] Except for saguaro and other cacti, life forms are grouped by height (sub-shrubs are <0.5 m, shrubs are 0.5 to 1.5 m, and trees are >1.5 m), and thus a single species may occur in multiple life form groups.

Table 3: One-year post glyphosate application percent green means separations for buffelgrass, life forms, and native non-target species under different glyphosate deposition rates and carrier volume combinations, listed by flight.

Analysis Group	Analysis Subgroup	Control	Flight 1A[a]	Flight 1B[a]	Flight 2A[a]	Flight 2B[a]
Buffelgrass	Open	76.8 a[b]	25.5 c	15.8 e	57.7 b	21.7 d
	Under Canopy	76.9 a	38.9 c	22.0 d	47.3 b	13.6 e
Life form	Other Cacti	83.4 a	85.2 a	79.2 a	79.8 a	86.0 a
	Saguaro	84.1 a	86.5 a	84.9 a	86.6 a	87.5a
	Shrub	76.2 a	75.9 a	76.6 a	74.1 a	57.2 b
	Subshrub	60.2 a	55.6 ab	44.8 bc	52.6 ab	41.9 c
	Tree	82.9 a	82.9 a	79.8 a	83.8 a	66.8 b
Species	mallow	10.9 a	10.3 a	5 a	28.8 a	5 a
	whitethorn acacia	79.6 a	66.1 a	56.7a	75.5 a	66.7 a
	triangle leaf bursage	69.1 a	67.5 a	49.5 ab	40.4 bc	25.0 c
	fairy duster	83.6 a	75.0 a	69.5 a	87.5 a	87.5 a
	staghorn cholla	84.4 a	87.5 a	79.3 a	75.7 a	87.5 a
	brittlebush	46.0 a	43.8 a	34.0 a	30.6 a	6.9 b
	barrel cactus	87.5 a	87.5 a	87.5 a	87.5 a	85.4 a
	ocotillo	87.5 a	82.7 ab	72.5 bc	78.8 ab	63.0 c
	limberbush	82.9 a	43.8 a	38.3 a	48.1 a	5 b
	slender janusia	70.6 a	77.5 a	60.8 a	68.0 a	69.2 a

Analysis Group	Analysis Subgroup	Control	Flight 1A[a]	Flight 1B[a]	Flight 2A[a]	Flight 2B[a]
	ratany	62.4 a	67.1 a	80.0 a	57.5 a	56.8 a
	wolfberry	83.7 a	83.2 a	83.0 a	84.0 a	62.2 b
	little leaf paloverde	83.3 a	85.6 a	82.4 a	86.1 a	69.8 b
	jojoba	78.0 a	87.5 a	83.4 a	81.9 a	87.5 a

[a] Glyphosate deposition on flights was as follows: 1A received 0.86 kg ae ha^{-1}, 1B received 1.65 kg ae ha^{-1}, 2A received 0.29 kg ae ha^{-1} , and 2B received 0.76 kg ae ha^{-1} glyphosate.

[b] Duncan's Multiple Range Test used to calculate means separations. Values within a column with same letters are not different ($P \leq 0.05$).

Table 4: Two-year post glyphosate application percent green means separations for life forms, and species under different glyphosate deposition rates and carrier volume combinations, listed by flight.

Analysis Group	Analysis subgroup	Control	Flight 1A[a]	Flight 1B[a]	Flight 2A[a]	Flight 2B[a]
Life form	Other Cacti	82.2 a[b]	84.3 a	76.9 a	81.5 a	87.5 a
	Saguaro	82.9 a	84.5 a	84.9 a	87.5 a	87.5 a
	Shrub	78.0 a	76.9 a	72.2 ab	74.1 ab	64.9 b
	Subshrub	67.4 a	58.1 ab	42.5 c	55.9 ab	47.5 bc
	Tree	83.2 a	83.8 a	82.7 a	85.6 a	72.7 b
Species	mallow	13.5 a	32.8 a	5 a	25.0 a	5 a
	whitethorn acacia	77.2 a	63.2 a	46.7 a	81.5 a	66.7 a
	triangle leaf bursage	82.6 a	77.8 a	64.1 a	63.8 a	28.6 b
	fairy duster	83.7 a	70.0 a	57.0 a	77.5 a	57.5 a
	staghorn cholla	83.8 a	87.5 a	79.3 a	77.5 a	87.5 a
	brittlebush	39.3 ab	47.3 a	8.3 bc	19.4 abc	6.4 c
	barrel cactus	83.4 a	87.5 a	79.5 a	87.5 a	87.5 a
	ocotillo	86.5 a	80.4 a	72.8 a	81.3 a	29.0 b
	limberbush	82.9 a	43.8 a	5 b	55.0 a	5 b
	slender janusia	75.5 a	87.5 a	74.4 a	71.4 a	63.6 a
	ratany	78.5 a	57.5 a	64.4 a	57.5 a	69.3 a
	wolfberry	85.1 a	83.2 a	78.4 ab	79.3 ab	74.2 b

Analysis Group	Analysis subgroup	Control	Flight 1A[a]	Flight 1B[a]	Flight 2A[a]	Flight 2B[a]
	little leaf paloverde	82.6 a	85.5 a	83.4 a	84.8 a	79.1 a
	jojoba	80.0 a	87.5 a	84.8 a	79.8 a	85.0 a

[a] Glyphosate deposition on flights was as follows: 1A received 0.86 kg ae ha^{-1}, 1B received 1.65 kg ae ha^{-1}, 2A received 0.29 kg ae ha^{-1} , and 2B received 0.76 kg ae ha^{-1} glyphosate.

[b] Duncan’s Multiple Range Test used to calculate means separations. Values within a column with same letters are not different ($P \leq 0.05$).

Table 5: Regression coefficients from simple linear regression of visually estimated percent greenness on glyphosate deposition rate for buffelgrass, life forms and non-target species one year post helicopter application.

Analysis Group	Analysis Subgroup	Regression coefficient[a]	Lower 95% CL[b]	Upper 95% CL[b]
Buffelgrass	Open	-42.1	-44.8	-39.5
Life form	Other Cacti	NS[c]	-	-
	Saguaro	NS	-	-
	Shrub	NS	-	-
	Subshrub	-9.9	-15.5	-4.3
	Tree	-3.5	-7.1	0
Species	mallow	NS	-	-
	whitethorn acacia	NS	-	-
	triangle leaf bursage	-11.3	-22.2	-0.4
	fairy duster	-8.6	-16.5	-0.8
	staghorn cholla	NS	-	-
	brittlebush	NS	-	-
	barrel cactus	NS	-	-
	ocotillo	-8.9	-15.4	-2.5
	limberbush	-37.9	-61.5	-14.2
	slender janusia	NS	-	-
	ratany	NS	-	-

Analysis Group	Analysis Subgroup	Regression coefficient[a]	Lower 95% CL[b]	Upper 95% CL[b]
	wolfberry	NS	-	-
	little leaf paloverde	NS	-	-
	jojoba	NS	-	-

[a] Rate of change in percent visual greenness per kg ae ha^{-1} glyphosate deposited (slope of the regression line).

[b] Upper and lower confidence levels of the regression coefficient.

c Regression not significant at $P \leq 0.05$.

Table 6: Regression coefficients from simple linear regression of visually estimated percent greenness on glyphosate deposition rate for buffelgrass, life forms and non-target species two years post helicopter application.

Analysis Group	Analysis Subgroup	Regression coefficient[a]	Lower 95% CL[b]	Upper 95% CL[b]
Life form	Other Cacti	NS[c]	-	-
	Saguaro	NS	-	-
	Shrub	NS	-	-
	Subshrub	-14.1	-20.1	-8.0
	Tree	NS	-	-
Species	mallow	NS	-	-
	whitethorn acacia	-17.5	-32.1	-2.9
	triangle leaf bursage	-12.6	-22.7	-2.5
	fairy duster	-16.5	-28.7	-4.3
	staghorn cholla	NS	-	-
	brittlebush	NS	-	-
	barrel cactus	NS	-	-
	ocotillo	-11.4	-21.7	-1.1
	limberbush	-56.2	-77.4	-35.1
	slender janusia	NS	-	-
	ratany	NS	-	-
	wolfberry	-4.1	-7.7	-0.4

Analysis Group	Analysis Subgroup	Regression coefficient[a]	Lower 95% CL[b]	Upper 95% CL[b]
	little leaf paloverde	NS	-	-
	jojoba	NS	-	-

[a] Rate of change in percent visual greenness per kg ae ha^{-1} glyphosate deposited (slope of the regression line).

[b] Upper and lower confidence levels of the regression coefficient.

c Regression not significant at $P \leq 0.05$

Figures

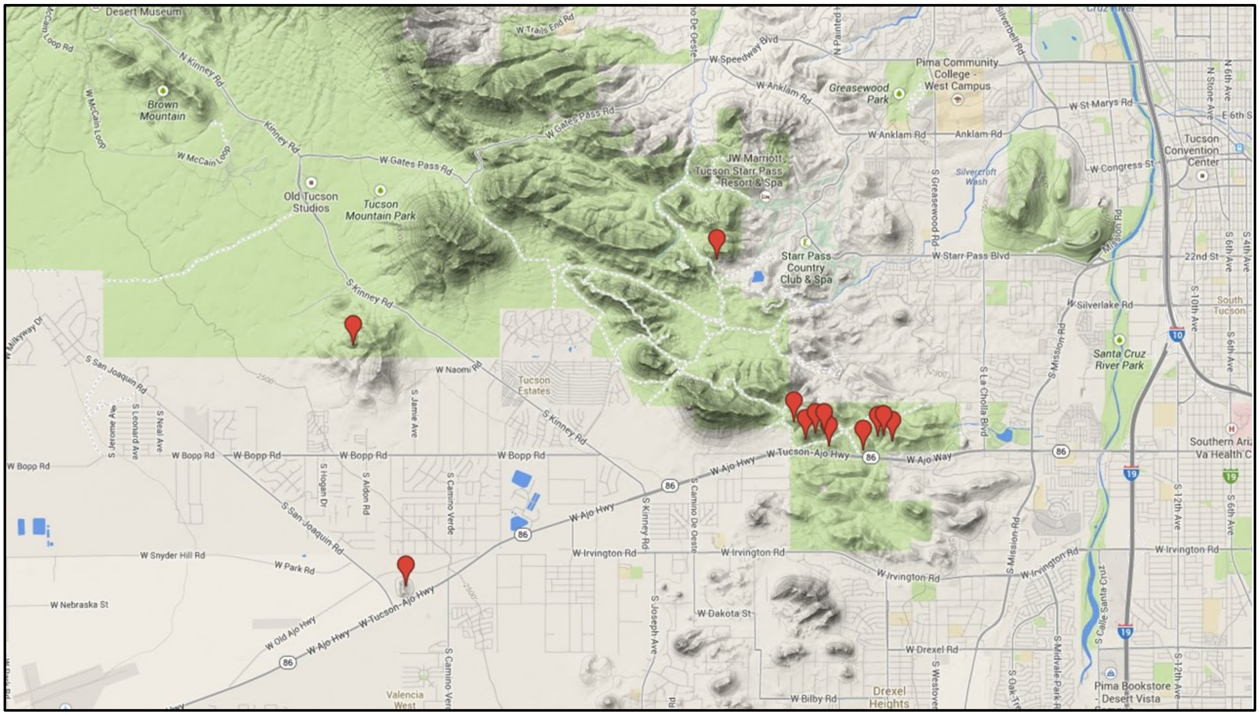

Figure 1: Relative locations of the 12 study plots in the southwestern Tucson Mountains.