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Thesis

LONG-TERM EFFECTS OF HERBICIDE TREATMENTS ON SPOTTED KNAPWEED AND
NON-TARGET PLANTS
IN THE GRAND SABLE DUNES

by

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ABSTRACT

Over 80 hectares of the Grand Sable Dunes in Pictured Rocks National Lakeshore has been heavily invaded by spotted knapweed (*Centaurea stoebe*). This species' propensity to dominate plant communities and stabilize active dunes creates the need for management in order to protect the globally rare perched dune ecosystem and its unique inhabitants. Milestone® and Transline® herbicides were spot sprayed on research treatments of knapweed for 5 consecutive years. Herbicide treatments were discontinued in some areas, and the recovering areas were compared to areas never invaded by knapweed. Herbicide treatments continued to be effective in controlling spotted knapweed populations; neither herbicide was significantly better at control of knapweed nor significantly more harmful to native species. Treatments recovering from herbicide generally were not significantly different from herbicide treatments, though some showed early anecdotal signs of recovery or reinvasion by spotted knapweed. Continued monitoring of this site is highly suggested for increased understanding of long-term effects of herbicide treatments.

Keywords: *Centaurea biebersteinii*, *Centaurea stoebe*, *Centaurea maculosa*, Milestone, Transline, dunes, Great Lakes region

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INTRODUCTION

It has been known for some time that invasive species alter the ecosystems they enter (Walker and Vitousek 1991, Legee & Murphy 2001), although quantifying this change can be very difficult due to lack of prior data (Vitousek and Walker 1989, Mack and D'Antonio 1998, National Research Council 2002). These impacts can be severe, whether the invader crowds or shades out natives or chemically or physically alters the environment (DiTomaso 2000, Pimentel *et al.* 2000). To combat these harmful effects, land managers attempt to decrease invasive abundance to low, less-harmful levels that can be maintained through management (Sheley *et al.* 1998).

Control of invasive plants usually involves either removal of individuals or killing the plants and leaving them in place. Physical removal, such as cutting, digging, or pulling out invasives can be effective, but is time and labor intensive, and may disturb existing seedbanks, uproot natives, or create opportunities for new invasive species to establish. Herbicide applications kill plants where they stand and leave seedbanks intact whereas prescribed burns may set back some non-native invasives and/or shift competition to favor native species adapted to fire. Herbicide treatments, in particular, can be aimed at specific plants, physically and chemically (Groves 1989, Nuzzo 1991), and are the focus of this study.

As with any treatment, non-target plants are affected by the drift of herbicides applied to other plants (Bohn *et al.* 2008). Short-term physiological recovery of non-target plants is linked to the stage of plant development when it was exposed to herbicide (Follak and Hurlle 2004), and an increase in percent cover of non-target plants in years following has been recorded (Bohn *et al.* 2010). Previous long-term herbicide research was conducted in terms

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of cost-effectiveness rather than actual native community recovery or efficacy against the target plants (Lym and Messersmith 1990, Munier-Jolain *et al.* 2002).

Spotted knapweed (*Centaurea stoebe*), a member of Asteraceae from Eastern Europe, is very good at changing its environment to suit it (Lym and Zollinger 1992). Its roots exude the allelopathic chemicals catechin (a phenol antioxidant that acts as an herbicide) and cnicin (a sesquiterpene lactone toxic to plants), which reduce seed germination of plants that might compete with it (Kelsey and Locken 1987, Ridenour and Callaway 2001, Bais *et al.* 2003). Additionally, knapweed grows rapidly in disturbed areas due to its large taproot and latticing secondary roots, and can spread quickly within an area to crowd out natives. One plant can produce more than 1,000 seeds per year, which can be viable for more than 5 years (Davis *et al.* 1993, USDA 2011).

The Grand Sable Dunes are a globally rare perched dune system in Michigan's Upper Peninsula on the southern shore of Lake Superior (Anderton and Loope 1995). The dune community as a whole is made of approximately 6 dune habitats, defined by amount and type of sand movement: stabilized areas, slight sand deposition, medium sand deposition, heavy sand deposition, deflation areas, and gravel lags. Five vegetation communities—which often correlate roughly with a certain type of habitat due to native plant adaptation—have also been identified on the Grand Sable Dunes: active dunes, gravel lags, stabilizing dunes, pioneer woodlands, and second growth forests (Bach 1978). These dunes have been designated a Natural Research Area in Pictured Rocks National Lakeshore (PRNL), which now protects and facilitates study of the federally-threatened Pitcher's thistle (*Cirsium pitcheri*) and state-threatened Lake Huron tansy (*Tanacetum huronense*) that flourish there (PRNL 2007, Higman and Penskar 1999).

The habitats of the Grand Sable Dunes that are disturbed by the natural processes of shifting sands, such as the areas of slight deposition and deflation, are the perfect places for spotted knapweed to invade. The knapweed's rapid rate of reproduction and growth can cause drastic changes to physical disturbance regimes, effectively removing colonization niches that would normally be available to native plants. Its allelopathic chemicals can also kill or hinder growth and colonization by native dune plants. Surveys in 1973 revealed the presence of spotted knapweed in Pictured Rocks National Lakeshore, but only along roads; no knapweed was found in the Grand Sable Dunes (Read 1975). A second survey, conducted in 1975, noted the presence of knapweed in the dunes, but in no great numbers (Bach 1978). By 2003, spotted knapweed populations had covered a huge area, estimated to comprise 30 hectares of the central area of the Grand Sable Dunes (Marshall *et al.* 2008). In 2009, the knapweed infestation had more than doubled to 80 hectares (Latsch 2011). At present, an estimated 150 hectares of the Grand Sable Dunes are invaded by exotic species (Pictured Rocks National Lakeshore, *unpublished data*).

Attempts have been made to control spotted knapweed in a variety of ways. Burning has proved effective at controlling populations in many environments (MacDonald *et al.* 2007). However, the native vegetation of the open dune ecosystem is not adapted to recover after fire, is often unable to withstand the heat of the fire itself, and—due to its sparse cover—does not sustain a controlled burn well. Additionally, the possible carcinogenic nature of spotted knapweed fumes and the lack of funding and fire-trained personnel at PRNL makes this option unattractive (Leutscher, *personal communication*). Hand-pulling is used in the Grand Sable dunes, but the sheer number of spotted knapweed individuals combined with the possibility of disturbing *C. stoebe* seeds stored in the soil make it

implausible over large areas; this method is reserved for areas deemed sensitive, such as those infestations close to Pitcher's thistle plants. This leaves herbicide application as the major control method at PRNL (Grzesiak 2010).

The herbicide currently in use against spotted knapweed at PRNL is Milestone, an aminopyralid (40.6%). It is suggested for use in controlling thistles and knapweeds, especially in rangelands (Dow AgroSciences 2011a). The aminopyralid works as a growth regulator, binding to auxin receptors and disrupting physiological processes when translocated throughout the target plant; in addition, it usually remains in the top 30 cm of soil to control later-emerging plants (Sharma and Singh 2001, Hartzler 2006). The second herbicide evaluated, Transline (clopyralid 40.9%) is recommended for use in the control of thistles, among other "troublesome weeds" (Dow AgroSciences 2011b). Clopyralid is also a growth regulator disruptor in broadleaf plants (Rice *et al.* 1997, Hartzler 2006). Neither herbicide should be applied directly to water or where water is present on the surface of the soil.

Exotic plant management teams (EPMTs) have hand-pulled or chemically treated the satellites populations with the herbicide Milestone for several years (Leutscher, *personal communication*), though Transline was used more often during the 2012 field season. These control efforts varied in intensity due to varying crew membership and funding; from 2008 to 2012, 4 to 7 person crews have been assigned to treat spotted knapweed in the Grand Sable Dunes almost exclusively for 3 months of the year (June through August). Milestone treatments of 0.09% (3.7 mL/100 m², standard use) or Transline at 0.30% (0.5 L/100 m², standard use) are "spot-sprayed" so only the target plants are purposely sprayed with herbicide. Herbicide concentrate is combined with dye (Blazon) and a non-ionic surfactant

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(Impact, 90% ethyloxyated alkylaryl) when mixed for spraying. All spraying is done on foot from backpack hand-pump sprayers (Grzesiak 2010).

From 2008 to 2010, a study was conducted on the efficacy of two herbicides, Transline and Milestone, on spotted knapweed and on the non-target native members of the dune community spotted knapweed had invaded. This work revealed no significant difference between the herbicides in either efficacy or harm, although both herbicides killed native plants (Latsch 2011). No studies concerning recovery after herbicide application have been done previously in the Grand Sable Dunes.

The purpose of this study is threefold: to learn about the effects of long-term herbicide application on spotted knapweed, to observe the effects of long-term application of herbicide on non-target native dune plants, and to monitor the first years of recovery of native communities (compared to uninvaded areas) and re-colonization by knapweed of areas that are no longer treated with herbicide. Understanding how herbicides affect the plant communities at large, and also how the community changes after herbicides are no longer applied, will be very useful as future management decisions are made concerning the Grand Sable Dunes and other similar ecosystems.

METHODS

Field Methods

Within an area of the Grand Sable Dunes that is extensively invaded by spotted knapweed, there exist six 19 m x 33 m blocks established during a study conducted from 2008 to 2010 (Latsch 2011, Fig. 1) and permanently marked in each corner with rebar and metal tags. Each block contains fifteen plots, divided among five repetitions of three treatments in random order: application of Milestone, application of Transline, and control (no herbicide). Each plot measured 5 m x 5 m, with a 2 m buffer established between each treatment area (Figure 2). Herbicides were applied—or not applied—to plots according to their assigned treatments once per year since summer 2008. The four plots of each treatment with the lowest percent cover of spotted knapweed in the summer of 2011 had herbicide application discontinued for 2011 and 2012.

Five 1 m² quadrats of vegetation were established within each plot. Percent cover was quantified using ocular measurements, and number of individuals rooted within the plot was hand counted for each species. In keeping with previous research (Latsch 2011), grasses and sedges (as “graminoids”), and mosses were counted as groups and not identified to species. Mosses were only quantified by percent cover. Quadrats were centered within each plot to avoid edge effects.

The treatment areas then had herbicides applied as appropriate for each area (Milestone, Transline, or no herbicide), with herbicides and personnel provided by PRNL. Treatments took place during July 2011 and 2012, when the spotted knapweed is just breaking buds (Latsch 2011). PRNL herbicide application procedures were followed,

including using a spot-spray method. A re-quantification of plant life in all areas was completed one week after treatment to give an estimation of immediate mortality.

In addition to the study areas set down by Latsch (2011), six new 5 m x 5 m study areas (“benchmark plots”) were established in a more western portion of the Grand Sable Dunes (Fig. 1), in which spotted knapweed populations are more limited, and where it is possible to find native dune ecosystems as they were before knapweed invasion. These “benchmark” areas contain no spotted knapweed, as few other non-native species as possible, and are in an area that is currently in a similar dune habitat (stabilized areas) and vegetation community (stabilizing dunes) to the existing study blocks. The benchmark plots were permanently marked with rebar and metal tags inscribed with plot number (“BM#”) and “UM-NPS” in each northeast corner; GPS points were also recorded. These plots were evaluated in the same way as the treatments, though they were not treated with herbicide and were only evaluated once per season.

Statistical Methods

A log transformation was used for graminoid and spotted knapweed count data, as some treatments had very high numbers, resulting in an exponential relationship between means and variances. Statistical analyses were performed using Microsoft Excel 2007 (Microsoft 2006) and R 2.12.1 (R-Project 2012).

For each treatment in each of the five years of the study, species richness, Shannon-Wiener diversity, and species evenness were calculated. Richness was calculated as the count of species or species groups in a given treatment. Shannon-Wiener diversity was calculated concerning both count of individuals and percent cover of the species or species group using the equation:

$$\mathbf{H} = -\sum p_i \ln p_i$$

Where p_i indicates percent cover or number of individuals of a given species (Hayek and Buzas 1997). Species evenness (Hayek and Buzas 1997) was calculated using both Shannon-Wiener diversity (\mathbf{H}) and species richness (\mathbf{S}) using the equation:

$$\mathbf{E} = \mathbf{H}/\ln(\mathbf{S})$$

In order to determine if there were differences between the four treatments in the 2011-2012 seasons, a Randomized Complete Block Analysis of Variance (ANOVA) was used. Independence was assumed for replications (plots), as assignment of the treatment for each plot was random within a block.

RESULTS

Response to herbicide

Spotted knapweed response—Generally speaking, *C. stoebe* populations decreased after being sprayed with either herbicide, (whether populations were quantified using count of individual plants or percent cover), while the spotted knapweed in control treatments remained the same. Knapweed populations also decreased in herbicide-treated areas over the five years of the study (Fig. 2), though there was a drastic increase in spotted knapweed counts in all treatment types in 2011 and 2012 compared to the previous three years.

In all cases 2011-2012, the Randomized Complete Block ANOVA and Tukey's Honestly Significant Difference Multiple Comparison tests reported significant differences in knapweed populations between the control and any other treatment ($p < 0.05$). When the

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ANOVA was calculated using mean percent cover, significant differences between knapweed populations treated with herbicide and those left to recover were significant in 2012 both before and after herbicide treatment, while these means were only significantly different in 2011 before herbicide treatments; after treatments in that year there was no significant difference between herbicide and recovery means. There was no significant difference between the Milestone and Transline treatments in either year whether measured by counts or percent cover of knapweed. When comparing *C. stoebe* counts, only the control was significantly different from other treatments.

Diversity, richness, and evenness response—A total of 30 species were located in the quadrats in 2011 and 2012 (Table 1), an increase of 5 species from the previous 3 years. Some new species were tree or shrub seedlings from nearby adults (*Pinus banksiana*, *Acer saccharum*, *Prunus virginiana*), one was invasive (*Taraxacum officinale*), and one was a “new” native plant (*Artemisia campestris*). Count and percent cover varied between treatments with some areas supporting a large number of plants (*e.g.* 660 individuals in Plot 5 of Block 12 to merely 14 in Plot 1 of the same Block), though graminoids and non-native invasives often had the highest numbers (*e.g.* an 80% cover of graminoids in Plot 4 of Block 12, versus 1% for dune cherry (*Prunus pumila*) and 40% cover for spotted knapweed in the same Plot).

Though species diversity declined after herbicide treatments, there was no difference between the two herbicide treatments, whether quantified by counts or percent cover. Species diversity in the control treatment was not different from the two herbicide treatments. When only native dune species were included in this metric, the small changes were more obvious (Fig. 3). The difference between treated and untreated areas, or areas treated with

the two herbicides was still not significant, though Milestone-treated treatments exhibited lower Shannon-Weiner diversity than Transline treatments for all five years when diversity was calculated using percent cover.

Mean species richness also exhibited no significant difference between herbicide treatments, or between these treatments and the control treatment. Though species richness decreased after spraying for each year, it was quite a small difference (*e.g.* from 4.8 to 4.4 for Milestone in 2008). These same patterns held true when only native dune species were included in the computation of species richness.

Mean species evenness calculations showed large differences between treatment and control for both count and percent cover, especially in 2009 (the second year of treatment); however, this difference decreased in each of the following years. The difference between Milestone- and Transline-treated areas is negligible overall, though Milestone-treated areas have lower species evenness in most cases. Similar patterns were observed when only native species were used to calculate evenness (Fig. 4), though at nearly half the scale.

Additionally, differences between Milestone and Transline treatments were more pronounced in native evenness calculated with percent cover.

Recovery from herbicide

Spotted knapweed populations where herbicide treatments were discontinued in 2011 (recovery plots) followed very similar trends to other herbicide-treated populations for the first three years, when herbicides were still in use (Fig. 5). In 2012, however, the spotted knapweed populations began to increase. The magnitude of this change differed between percent cover and number of individuals, though there did not appear to be much difference between treatments that had originally been treated with Milestone or Transline.

Benchmarks, in which spotted knapweed never established, had a smaller number of species than the invaded areas but also fewer invasives (Table 2). Eighteen species were found, one of which was non-native (hawkweed, *Hieraceum* spp.). Three native species were present in the benchmarks, but not in the knapweed-invaded areas: Pitcher's thistle (*Cirsium pitcheri*), beach heath (*Hudsonia tomentosa*), and sandbar willow (*Salix exigua*).

Shannon-Weiner diversity in the recovery communities dropped, then slowly rose during years of herbicide use, whether mean diversity was calculated using count or percent cover (Fig. 6). When herbicide treatments were discontinued, overall diversity calculated using number of individuals held steady at a very similar index value to that calculated for the first year, before treatment. However, diversity of the recovery plant populations was still much lower than that of the benchmarks, especially when diversity was calculated using percent cover. When only native species were considered, Shannon-Weiner diversity was much lower, didn't change as much initially, and increased above the initial 2008 diversity index, whether calculated with counts or percent cover (Fig. 6). Herbicide recovery population means that had been treated with Transline previously showed greater and more rapid increase in native diversity, though neither Milestone nor Transline recovery treatments reached diversity values as high as the uninvaded benchmarks.

Overall species richness in the recovery followed a similar overall pattern to that exhibited by overall Shannon-Weiner diversity calculated using percent cover, including Transline treatment species richness increasing faster and more than that of Milestone treatments (Fig. 7). However, benchmark richnesses were not greater than those of recovery treatments; in both 2011 and 2012, Transline-treated recovery areas had higher mean species richness than the benchmarks, and Milestone-treated recovery areas had equal richness to

benchmarks in 2011. When only native species were used in calculations, there was less change in mean richness during years that the recovery treatments were treated with herbicides, though Transline-treated areas possessed slightly higher richness for each of the three years of treatment (Fig. 7). When herbicide treatment stopped, species richness increased for all recovery areas, though their indices remained slightly lower than that of the benchmark.

Species evenness—including all present species—exhibited similar changes to that of diversity (when calculated using percent cover), whether evenness was calculated using count or percent cover; there was no significant difference in evenness between the two herbicide treatments. The same held true when evenness calculations incorporated only native dune species.

Finally, wild strawberry (*Fragaria vesca*), Lake Huron tansy (*Tanacetum huronense*), goldenrod (*Solidago* spp.), hairy puccoon (*Lithospermum caroliniense*), starry false Solomon's seal (*Smilacina stellata*), dune cherry (*Prunus pumila*), and common evening primrose (*Oenothera parviflora*) are common members of the dune community present for comparison between Transline- and Milesone-treated recovery areas, as well as with the benchmarks. Additionally, the species represented a variety of growth habits: biennial herb, (primrose), clonal spreading shrub (dune cherry), trailing herbaceous perennial (strawberry), and various perennials. When percent cover was used, the tansy and puccoon saw similar trends in population between the two types of recovery treatments, while the strawberry and Solomon's seal saw opposing trends between the two; goldenrod, cherry, and primrose populations showed somewhat similar patterns between types of recovery (Fig. 8). Most species' populations are lower than the average for the benchmarks, though Solomon's seal

populations are greater in recovery treatments than benchmarks overall, and dune cherry is more common in Transline-treated recovery treatments.

When counts are considered instead of percent cover, trends are generally smoother, with less opposition in trends between the two herbicide treatments, though there are more single-point spikes (Fig. 9). Solomon's seal populations in recovery treatments are still mostly greater than the mean benchmark population, and strawberry and goldenrod only spike above the average in 2011's Transline-treated recovery treatments.

DISCUSSION

Response to herbicide

Spotted knapweed response—The decrease in *C. stoebe* populations following herbicide treatments continues the pattern found by Latch (2011). The abrupt increase in spotted knapweed density in 2011 (Fig. 3) could have been due to the change in researchers making observations, though the fact that one field researcher remained the same for all five years and that the percent cover estimations remain relatively steady makes this somewhat unlikely. Regardless, the fact that knapweed populations fall to near zero each time they are sprayed, and stay low (a fraction of the original population, and of the control populations) in the following year even before they are treated indicates that both herbicides are effective at spotted knapweed control, even for as long as 5 years. The continuation of no significant difference between the two herbicides, and significant differences between treated and control knapweed populations also followed and supported the findings of Latch (2011). This indicates that both herbicides are equally suited for use in control of *C. stoebe*, at least in regards to mortality and regeneration from year to year.

Diversity, richness, and evenness response—The apparent recovery of overall diversity of herbicide-treated areas in the last 3 years of the study is promising for restoration efforts, but does not tell the whole story. Native diversity, when quantified by count, does not change so drastically as overall diversity, and most times reports no significant difference between control and treated means (Fig. 3). This indicates that there may be very little effect on native species by the herbicides, hopefully due to excellent spray practices. Quantifying native diversity using percent cover shows more differences between in means, specifically between the two herbicides; native plant diversity in areas treated with Milestone was always lower than that of Transline treatments, and in some cases (2010, 2012), the differences may be significant, though neither was significantly different from the control (Fig. 3). This could indicate stunting of native plants by the herbicide Milestone; further research in this area is suggested. These differences from overall diversity indicate that invasion by other non-native species may be occurring with the decrease in spotted knapweed populations, which is supported by the notation of a new non-native, *Dianthus armeria*, in 2010 (Latch 2011).

Similar conclusions can be drawn concerning the decline in 2009 in overall species richness compared with the small amount of any change in native species richness. The minute changes and overall steady state of average number of species indicates that very little is changing in the native community with herbicide application, meaning neither herbicide has much of a negative effect when applied in this manner.

The fact that overall evenness was nearly twice native evenness (Fig. 4) was not in and of itself surprising. Graminoids, a group of plants with the largest count and percent cover, are native plants, while most other natives have comparatively tiny abundances. This would result in a very low evenness, especially when compared to the overall score, which

would include such “high-scoring” plants as spotted knapweed, hawkweed (*Hieracium* spp.), and red sorrel (*Rumex acetosella*). These non-natives would increase the evenness score significantly. It may be worth noting, however, that the low evenness is not necessarily desirable, as uninvaded habitats have much greater evenness.

Recovery from herbicide

The fact that, when considering percent cover, recovery population mean shows significant difference from that of the controls and no significant differences from the herbicide-treated populations in 2011 after herbicide application indicates that overall, recovery treatments have retained the low-levels of knapweed attained by 3 years of herbicide application. When knapweed individual counts are considered instead, the lack of significant differences between recovery and herbicide-treated means is even more uplifting; the fact that there are not significantly more individuals in an area that hasn't been treated with herbicide for two years than an area that has been treated the entire time indicates efficacy for these treatments in the long-term. The idea that heavily invaded areas can be treated for as little as 3 years before they enter a “maintenance phase” could save land managers money and time, as such populations would simply need monitoring for at least a few years. The fact that these blocks were at most a few meters from heavily infested areas that could be seeding the area in question and still maintained their low populations is encouraging.

The difference between recovery and herbicide knapweed mean percent cover before herbicide application in 2011 can be explained by the fact that recovery treatments were chosen specifically because they had the lowest knapweed abundance of the given block; it is not a stretch to guess that this would make the recovery populations significantly different

from those of the treatments before the latter were sprayed with herbicide. It is also logical, then, that the lack of significant difference between recovery and herbicide-treated *C. stoebe* populations after treatment in 2012, as the herbicide would have brought the means of Milestone- and Transline-treated knapweed lower to match that of the low-population recovery.

The tight similarities in the population curves of spotted knapweed populations continually sprayed with either Milestone or Transline and those now in recovery (Fig. 5) is an excellent visual representation of the Tukey test's conclusions; the populations of knapweed decrease together, spike, then continue to decrease in a very similar manner. However the difference between recovering and treated populations in 2012 as portrayed by percent cover could be important, as the recovery shows a more marked increase in knapweed cover. However, as 2012 is the first year recovery areas received no treatment in the previous season, it is not conclusive. Continuing such a long-term study is highly suggested and highly sought-after.

The fact that overall diversity in recovering plant populations stayed relatively the same in the two years is heartening, as native diversity also remained similar (Fig. 6); therefore, few invasives might have returned or increased in population, and few natives were deeply impacted. Unfortunately, diversity did not increase in this short timespan either; the diversity in the benchmarks remains much higher. The decline of overall species richness combined with the slight increase of native species richness (Fig. 7) during recovery years suggests that more time will allow diversity to increase as well, given more time for reproduction of native plants. In the same vein, the large gap between recovery and benchmarks for both overall and native species evenness indicates that more time is needed

for adequate recovery of the dune ecosystem. As before, further study is needed in order to create a more reasonable picture of recovery as well as to provide clues as to which herbicide allows natives to recover better.

The population fluctuations demonstrated by the native species in treated areas chosen as models (Figs. 8, 9) appear convoluted, suggesting that these plants were impacted more by their own species dynamics than by herbicide treatments, or the cessation of the same. However, the slight increase seen in 2011 and/or 2012 for most species is encouraging. Continued research on this site should yield very interesting and applicable results.

CONCLUSIONS

Shortcomings

Two major shortcomings stand out in this research: the short timeline for a long-term study, and the location of the recovery plots. The small amount of time in which this study was conducted, especially the recovery portion, means few data points and always the cry for further study. To truly make measured judgments and recommendations regarding long-term efficacy of herbicides, especially concerning cessation of treatment, a minimum of two more years of study is necessary; more is certainly ideal.

The location of recovery plots—more specifically, that the recovery plots are located directly adjacent to buffer zones and control plots and within a relatively large valley that is highly invaded by knapweed—means that there is a huge volume of new *C. stoebe* seeds readily available to re-colonize and re-invade. This would not be true of areas that were truly treated for spotted knapweed; invasive species control efforts treat the entire site, to minimize

adult knapweeds literally a few centimeters away to confound efforts. While this problem did not appear to directly manifest itself in the two years of recovery analysis, there is a strong possibility that it will become more of a confounding factor if the study continues.

Herbicide efficacy

Overall, the two herbicides both proved to be effective at controlling *C. stoebe*, as Latch (2011) and many others concluded. Neither Milestone nor Transline has been more effective than the other at controlling populations, nor does one impact the native ecosystem more negatively than the other, on the whole. Despite this continued positive response, further long-term study on this site is very strongly recommended. Not only is such work rare, but knowing more about how prolonged use of herbicides affects the target plant is extremely valuable both to science and natural areas management. Additionally, it may be only in the long-term that a difference between the two herbicides appears.

Recovery

Again, neither herbicide appears to have a stronger effect on either the recovery of native species, or the reestablishment of invasive populations. However, with only two years of data available, and with only one of those years having not been treated in the previous season, it is difficult to truly draw conclusions. Another 2 years—minimum—of research is highly recommended in order to better understand how ecosystems change and recovery after invasives have been controlled. This information would have a vast impact not only on management choices, but on future research.

Recommendations

Based on this study, natural area managers should choose between the aminopyralid and triclopyr herbicides for *Centaurea stoebe* control based on soil type and water tables—as recommended by the pesticides’ labels—and budgetary constraints. More importantly, decision-makers should consider putting areas which have had spotted knapweed controlled using one or both of these herbicides into a “maintenance phase.” This study reveals that at least two years is a reasonable rest period with no major rebound in knapweed populations. During this time the area should be monitored for knapweed resurgence, but the cost—in money, time, and prospective resistance to the pesticide by the knapweed—should be much less, allowing for other projects to be prioritized. This also allows seedlings time to mature into rosettes or even bolt. Though that idea is not desirable in and of itself, these larger plants are easier for surveyors and herbicide applicators alike to find and therefore manage.

REFERENCES

- Anderton, J. B. and W. L. Loope. 1995. Buried soils in a perched dunefield as indicators of late Holocene lake-level change in the Lake Superior basin. *Quatern. Res.* 44:190–199.
- Bach, David P. 1978. MS Thesis. Plant communities, habitats, and soil conditions of Grand Sable Dunes, Pictured Rocks National Lakeshore, Michigan. Michigan Technological University; Houghton, MI.
- Bais, H.P., R. Vepachedu, S. Gilroy, R.M. Callaway, and J.M. Vivanco. 2003. Allelopathy and exotic plant invasion: from molecules and genes to species interactions. *Science* 301(5638) 1377-1380.
- Bohn, K. K., P. J. Minogue, J. McKeithen, A. Osieka and S. Jones. 2008, August 5. Impacts to non-target vegetation following herbicide control of the invasive Japanese climbing fern (*Lygodium japonicum*) in the forests of northwest Florida. Poster session presented at the 93rd Ecological Society of America Annual Meeting, Milwaukee, WI.
- Bohn, K. K., P. J. Minogue, J. McKeithen, and E. C. Pieterse. 2010, August 5. Cover, composition and diversity following herbicide treatment of the invasive Japanese climbing fern in pine forests of northwest Florida. Poster session presented at the 95th Ecological Society of America Annual Meeting, Pittsburgh, PA.
- Davis, E.S., P.K. Fay, T.K. Chicoine, and C.A. Lacey. 1993. Persistence of spotted knapweed (*Centaurea maculosa*) seed in soil. *Weed Science* 41:57-61.
- DiTomaso, J.M. 2000. Invasive weeds in rangelands: Species, impacts, and management. *Weed Science* March 2000, Vol. 48, No. 2, pp. 255-265.
- Dow AgroSciences. 2011a. Milestone herbicide. Dow AgroSciences LLC. Accessed 11 April 2011. <http://www.dowagro.com/range/products/milestone.htm>
- Dow AgroSciences. 2011b. Transline herbicide. Dow AgroSciences LLC. Accessed 11 April 2011. <http://www.dowagro.com/ivm/invasive/prod/trans.htm>
- Follak, S. and K. Hurlle. 2004. Recovery of non-target plants affected by airborne bromoxynil-octanoate and metribuzin. *Weed Research* 44:142–147
- Groves, R.H. 1989. Ecological control of invasive terrestrial plants. *Biological invasions*. J. Wiley and Sons, New York, New York, USA, 437-462.
- Grzesiak, K. 2010. Pictured Rocks National Lakeshore Exotic Plant Management Team Handbook.
- Hartzler, B. 2006. Aminopyralid—new herbicide for pastures, roadsides, etc. Iowa State University Extension Agronomy. Accessed 13 April 2011. <http://www.weeds.iastate.edu/mgmt/2006/aminopyralid.shtml>
- Hayek, L.C. and M.A. Buzas. 1997. Surveying natural populations. New York: Columbia University Press. 563.

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Thesis

- Higman, P.J. and M.R. Penskar. 1999. Special plant abstract for *Cirsium pitcheri*. Michigan Natural Features Inventory, Lansing, MI. 3 pp.
- Kelsey, R. G. and L. J. Locken. 1987. Phytotoxic properties of cnicin, a sesquiterpene lactone from *Centaurea maculosa* (spotted knapweed). *J. Chem. Ecol.* 13:19-33.
- Latsch, M. 2011. Doctoral Dissertation. Effects of management on native and exotic plant communities in Picture Rocks National Lakeshore in the Upper Peninsula of Michigan. Michigan Technological University; Houghton, MI.
- Leege, L.M. and P.G. Murphy. 2001. Ecological effects of the non-native *Pinus nigra* on sand dune communities. *Canadian Journal of Botany*, 79(4), 429-437.
- Lym, R. G. and C. G. Messersmith. 1990. Cost-effective long-term leafy spurge (*Euphorbia esula*) control with herbicides. *Weed Technology* 4:635-641.
- Lym, R. G. and R.K. Zollinger. 1992. Spotted knapweed (*Centaurea maculosa* Lam.). North Dakota State University Ext. Ser. Circ. W-842 (revised). Fargo, ND.
- MacDonald, N.W., B.T. Scull, and S.R. Abella. 2007. Mid-spring burning reduces spotted knapweed and increases native grasses during a Michigan experimental grassland establishment. *Restoration Ecology* 15:118-128.
- Mack, M.C. and C.M. D'Antonio. 1998. Impacts of biological invasions on disturbance regimes. *Trends in Ecology & Evolution*, 13(5), 195-198.
- Marshall, J.M., A.J. Storer, and B. Leutscher. 2008. Comparative analysis of plant and ground dwelling arthropod communities in lacustrine dune areas with and without *Centaurea biebersteinii* (Asteraceae). *The American Midland Naturalist* 159(2):261-274.
- Microsoft. 2006. Microsoft Office Excel 2007. User's Manual. Accessed 22 October 2012. download.microsoft.com/.../c/.../excel2007productguide.doc
- Munier-Jolain, N.M., B. Chavvel, and J. Gasquez. 2002. Long-term modelling of weed control strategies: analysis of threshold-based options for weed species with contrasted competitive abilities. *Weed Research*, 42(2), 107-122.
- National Research Council. 2002. Predicting invasions of nonindigenous plants and plant pests. National Academy Press, Washing, DC.
- Nuzzo, V.A. 1991. Experimental control of garlic mustard (*Alliaria petiolata* (Bieb.) Cavara & Grande) in northern Illinois using fire, herbicide, and cutting. *Natural Areas Journal*. 11: 158–167.
- Pictured Rocks National Lakeshore (PRNL). 2007. Grand Sable Dunes Research Natural Area. National Park Service. Accessed 13 April 2012. <http://www.nps.gov/PRNL/naturescience/upload/GrandSableDunesColor%202008.pdf>.
- Pimentel, D., L. Lach, R. Zuniga, and D. Morrison. 2000. Environmental and economic costs of nonindigenous species in the United States. *BioScience*, 50(1), 53-65.

K. Grzesiak

Thesis

- R-Project. 2012. The R Project for Statistical Computing. Accessed 7 July 2012. <http://www.r-project.org/index.html>
- Read, R.H. 1975. Vascular plants of Pictured Rocks National Lakeshore, Alger County, Michigan. *The Michigan Botanist* 14(1): 3-43.
- Rice, P.M., J.C. Toney, D.J. Bendunah, and C.E. Carlson. 1997. Plant community diversity and growth form responses to herbicide applications for control of *Centaurea maculosa*. *Journal of Applied Ecology* 34:1397-1412.
- Ridenour, W.M. and R.M. Callway. 2001. The relative importance of allelopathy in interference: the effects of an invasive weed on a native bunchgrass. *Oecologia* 126(3):444-450.
- Sharma, S.D. and M. Singh. 2001. Susceptibility of Florida candler fine soil to herbicide leaching. *Bulletin of Environmental Contamination and Toxicology* 67:594-600.
- Sheley, R.L., J.S. Jacobs, and M.F. Carpinelli. 1998. Distribution, biology, and management of diffuse knapweed (*Centaurea diffusa*) and spotted knapweed (*Centaurea maculosa*). *Weed Technology*, 353-362.
- United States Department of Agriculture, NRCS. 2011. The PLANTS Database. National Plant Data Center, Baton Rouge, LA, USA. Accessed 2 August 2012. <http://plants.usda.gov>.
- Vitousek, P.M. and L.R. Walker. 1989. Biological invasion by *Myrica faya* in Hawai'i: plant demography, nitrogen fixation, ecosystem effects. *Ecological Monographs*, 59(3), 247-265.
- Walker, L. R. and P. M. Vitousek. 1991. An invader alters germination and growth of a native dominant tree in Hawaii. *Ecology*, 72:1449-1455.

TABLES

Table 1: Plants located in the herbicide study quadrats in the Grand Sable Dunes, Pictured Rocks National Lakeshore, Michigan, 2011-2012. This table does not include species found in benchmark treatments (uninvaded by spotted knapweed). Status indicates “T” for state threatened species, “SC” for species of special concern, or “I” for invasive non-native plants.

Latin name	Common Name	Status
<i>Acer saccharum</i>	Sugar maple	
<i>Achillea millefolium</i>	Yarrow	
<i>Arabis lyrata</i>	Lyrate rockcress	
<i>Artemisia campestris</i>	Field sagewort	
<i>Asclepias syriaca</i>	Common milkweed	
<i>Botrychium spp.</i>	Moonwort	SC
<i>Centaurea stoebe</i>	Spotted knapweed	I
<i>Comandra umbellata</i>	Bastard toadflax	
<i>Dianthus armeria</i>	Deptford pink	I
<i>Equisetum arvense</i>	Field horsetail	
<i>Fragaria vesca</i>	Woodland strawberry	
<i>Hieracium spp.</i>	Hawkweed	I
<i>Juniperus communis</i>	Common juniper	
<i>Lathyrus japonicus</i>	Beach pea	
<i>Leucanthemum vulgare</i>	Ox-eye daisy	I
<i>Lithospermum caroliniense</i>	Hairy puccoon	
<i>Mianthemum stellatum</i>	Dune cherry	
<i>Oenothera parviflora</i>	Evening primrose	
<i>Pinus banksiana</i>	Jack pine	
<i>Plantago major</i>	Broadleaf plantain	I
<i>Poaceae etc.</i>	Graminoid	
<i>Prunus pumila</i>	Dune cherry	
<i>Prunus virginiana</i>	Choke cherry	
<i>Rosa blanda</i>	Smooth rose	
<i>Rumex acetosella</i>	Red sorrel	I
<i>Mianthemum stellatum</i>	Starry false Solomon seal	
<i>Solidago spp.</i>	Goldenrod	
<i>Stellaria longipes</i>	Longstalk starwort	SC
<i>Tanacetum huronense</i>	Lake Huron tansy	T
<i>Taraxacum officinale</i>	Dandelion	I
<i>Toxicodendron radicans</i>	Poison ivy	

Table 2: Plants located in benchmark quadrats (never any spotted knapweed) in the Grand Sable Dunes, Pictured Rocks National Lakeshore, Michigan, 2011-2012. Status indicates “T” for state-threatened species, “*T” for federally-threatened species, “SC” for species of special concern, or “I” for invasive non-native plants.

Latin Name	Common Name	Status
<i>Acer saccharum</i>	Sugar maple	
<i>Arabis lyrata</i>	Lyrate rockcress	
<i>Artemisia campestris</i>	Field sagewort	
<i>Cirsium pitcheri</i>	Pitcher's thistle	*T
<i>Fragaria vesca</i>	Woodland strawberry	
<i>Hieracium spp.</i>	Hawkweed	I
<i>Hudsonia tomentosa</i>	Beach heath	
<i>Lathyrus japonicus</i>	Beach pea	
<i>Lithospermum caroliniense</i>	Hairy puccoon	
<i>Oenothera biennis</i>	Evening primrose	
<i>Poaceae etc.</i>	Graminoids	
<i>Prunus pumila</i>	Dune cherry	
<i>Salix exigua</i>	Sandbar willow	
<i>Mianthemum stellatum</i>	Starry false Solomon seal	
<i>Solidago spp.</i>	Goldenrod	
<i>Stellaria longipes</i>	Longstalk starwort	SC
<i>Tanacetum huronense</i>	Lake Huron tansy	T
<i>Toxicodendron radicans</i>	Poison ivy	

FIGURES

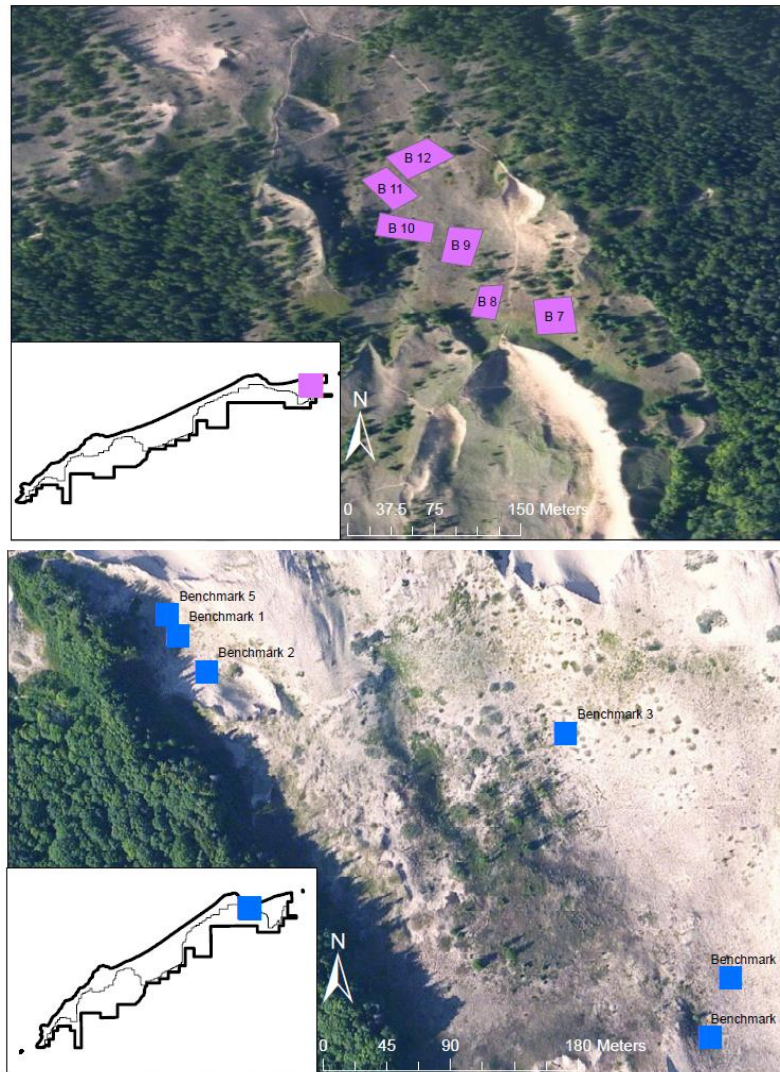


Figure 1: Placement of existing study blocks (top) and of benchmarks (bottom, where spotted knapweed has never invaded) in the Grand Sable Dunes, Pictured Rocks National Lakeshore, Michigan.

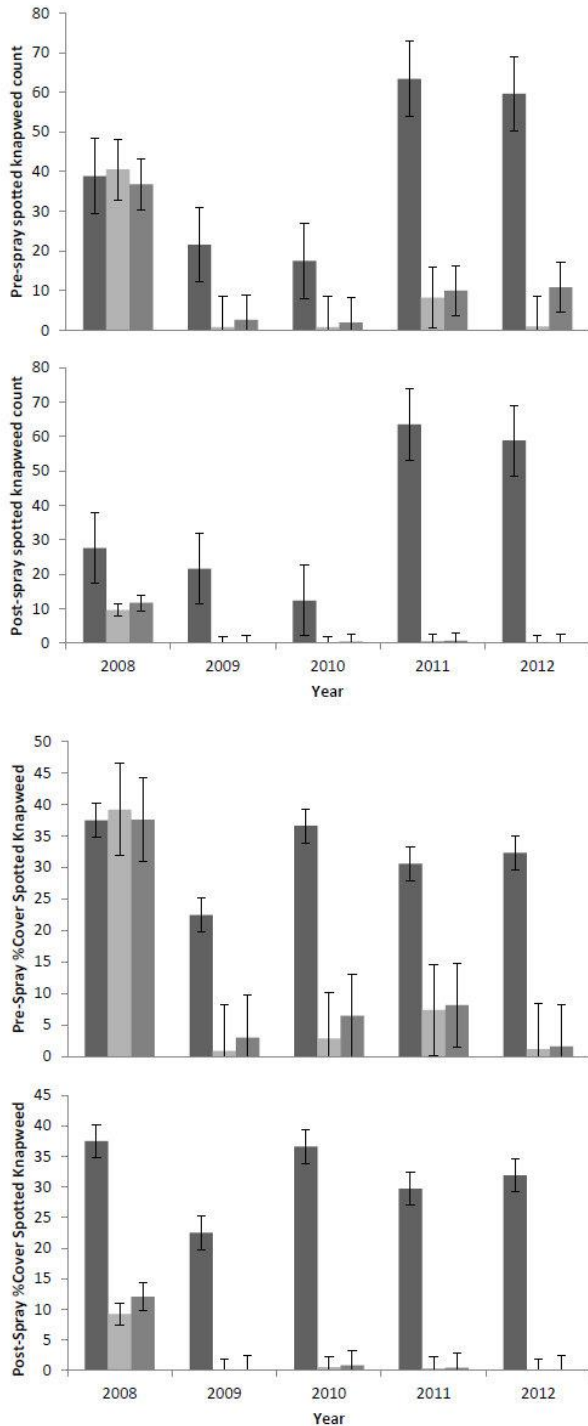


Figure 2: Average spotted knapweed (*Centaurea stoebe*) populations, quantified by number of individual plants or percent cover, using before (pre-spray) and after (post-spray) herbicide applications over five years in the Grand Sable Dunes, Pictured Rocks National Lakeshore, Grand Marais, MI. The darkest columns represent control treatments, the lightest were treated with Milestone herbicide, and the medium-darkness columns reflect those treatments sprayed with Transline herbicide.

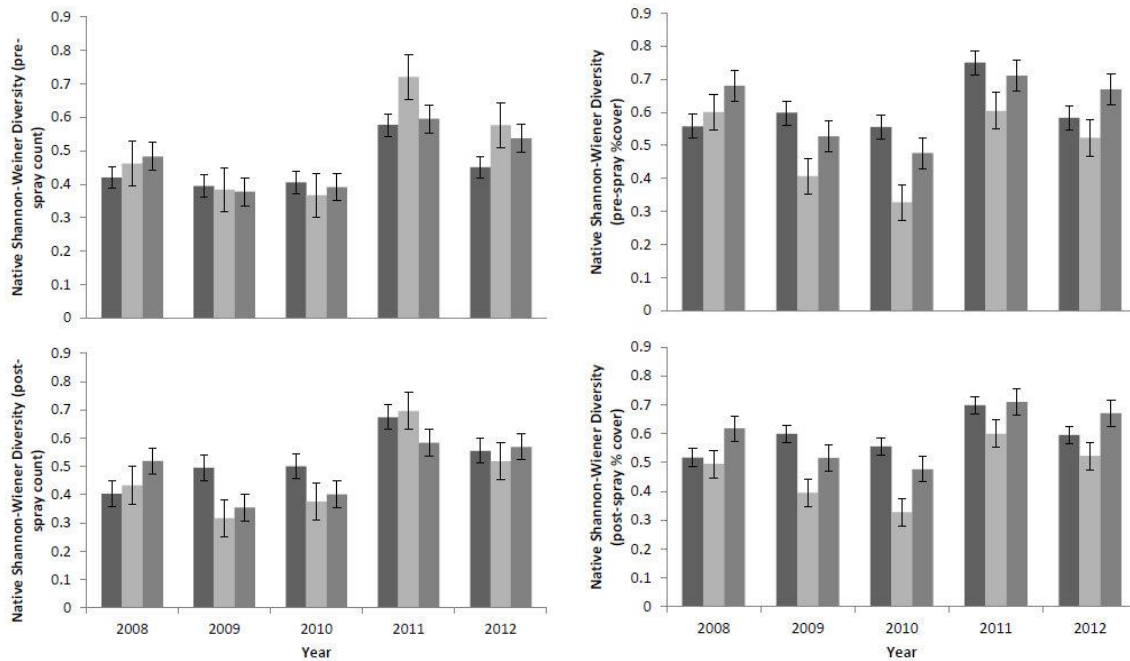


Figure 3: Average Shannon-Weiner diversities of plants native to Great Lakes dunes, calculated using number of individuals or percent cover, before (pre-spray) and after (post-spray) herbicide applications over five years in the Grand Sable Dunes, Pictured Rocks National Lakeshore, Grand Marais, MI. The darkest columns represent control treatments, the lightest were treated with Milestone herbicide, and the medium-darkness columns reflect those treatments sprayed with Transline herbicide.

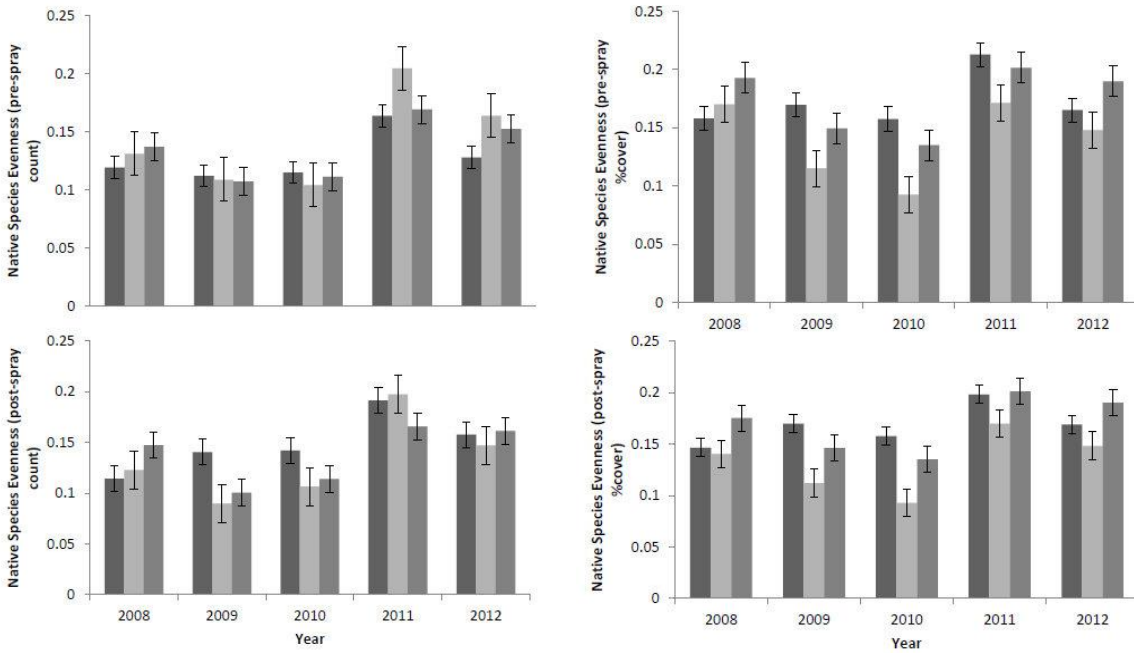


Figure 4: Average species evenness of native dune plants, calculated using counts of individuals or percent cover, before (pre-spray) and after (post-spray) herbicide applications over five years in the Grand Sable Dunes, Pictured Rocks National Lakeshore, Grand Marais, MI. The darkest columns represent control treatments, the lightest were treated with Milestone herbicide, and the medium-darkness columns reflect those plots sprayed with Transline herbicide.

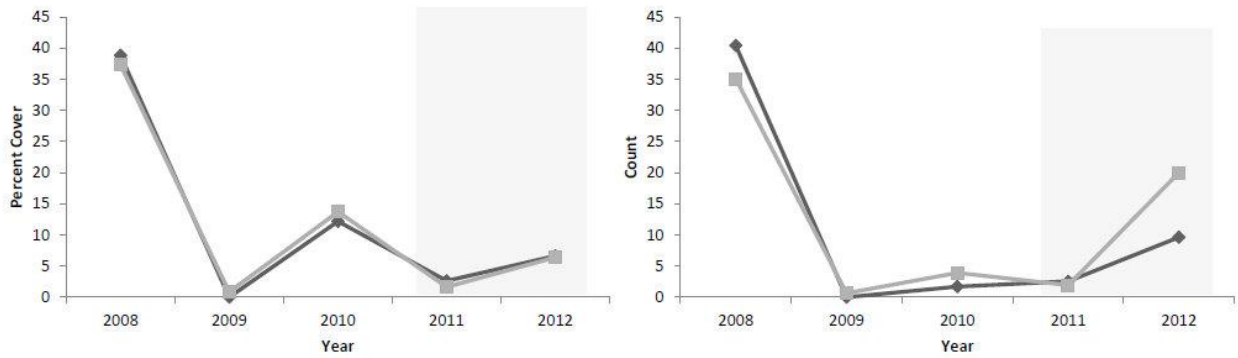


Figure 5: Spotted knapweed (*Centaurea stoebe*) population trends with herbicide application and cessation (“recovery”) over five years in the Grand Sable Dunes, Pictured Rocks National Lakeshore, Grand Marais, MI. The diamond-points and dark lines represent treatments sprayed with Milestone and the lighter square-points and lines represent treatments sprayed with Transline. The light grey box shading years 2011 and 2012 reflects when the treatments were not sprayed with any herbicide.

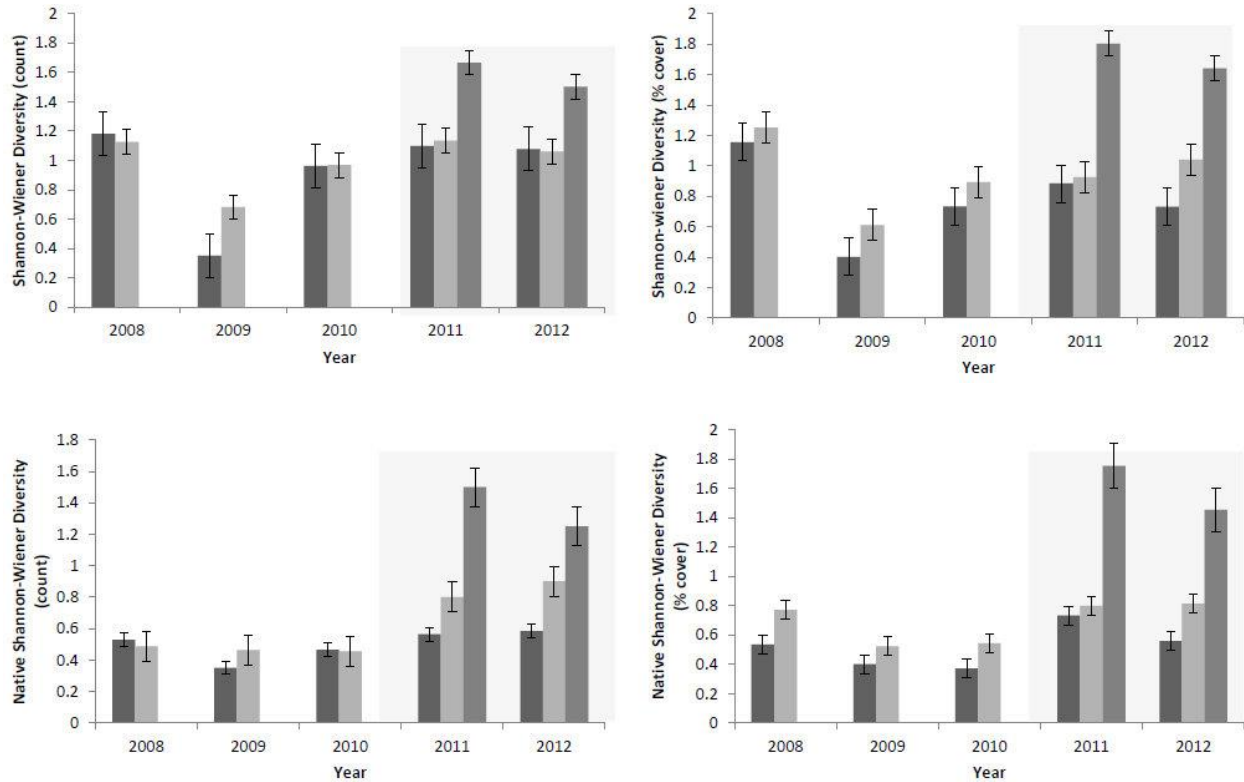


Figure 6: Average Shannon-Weiner Diversity, calculated using number of individuals or percent cover, with herbicide application and cessation (“recovery”) over five years in the Grand Sable Dunes, Pictured Rocks National Lakeshore, Grand Marais, MI. The top two graphs portray all species in the treatments, while the bottom two were calculated using only species native to Great Lakes dunes. The darkest columns represent treatments sprayed with Milestone, the lightest columns represent treatments sprayed with Transline, and the medium-grey columns stand for benchmarks (plots in an area that has never had spotted knapweed). The light grey box shading years 2011 and 2012 reflects when the Milestone and Transline treatments were not sprayed with any herbicide.

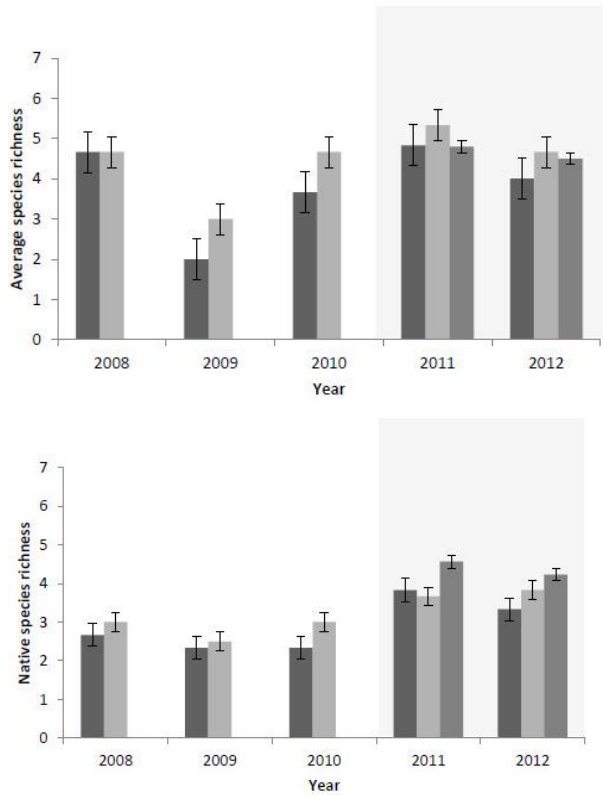


Figure 7: Average species richness, of all plants or only those native to Great Lakes dunes, with herbicide application and cessation (“recovery) over five years in the Grand Sable Dunes, Pictured Rocks National Lakeshore, Grand Marais, MI. The darkest columns represent treatments sprayed with Milestone, the lightest columns represent treatments sprayed with Transline, and the medium-grey columns stand for benchmarks (plots in an area that has never had spotted knapweed). The light grey box shading years 2011 and 2012 reflects when the Milestone and Transline treatments were not sprayed with any herbicide.

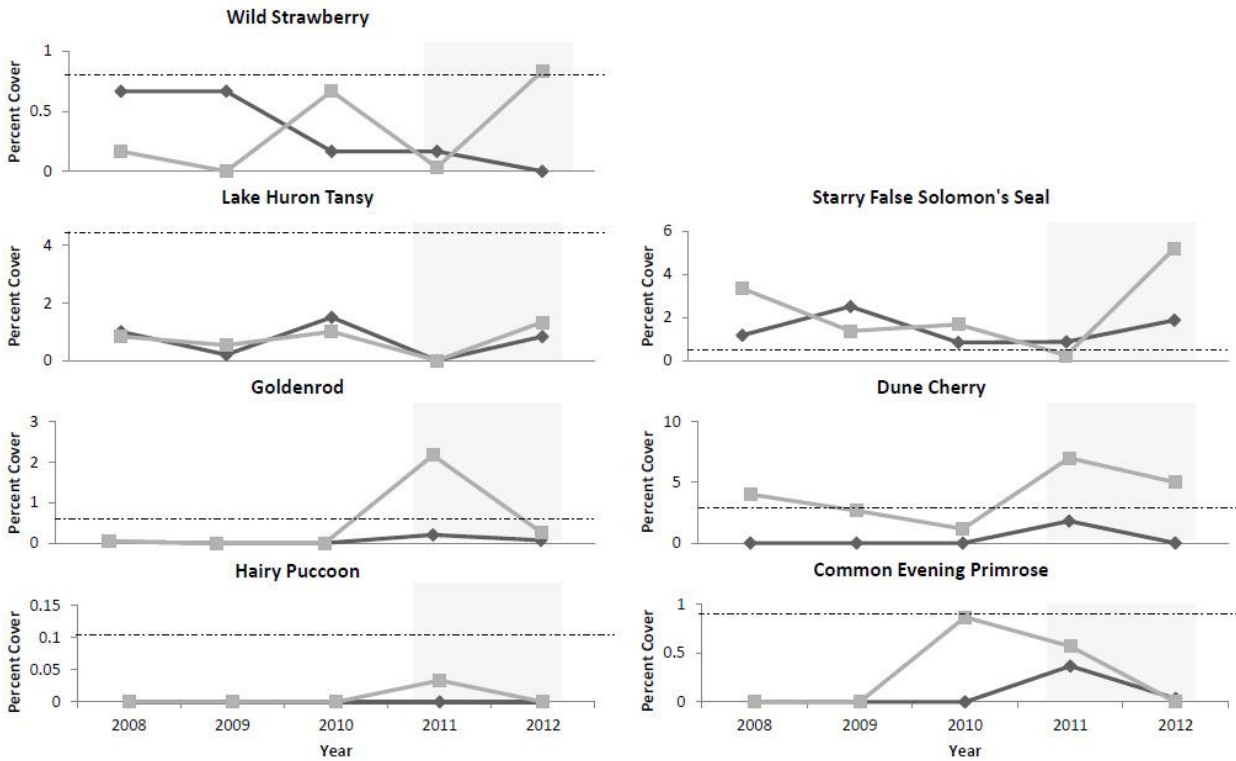


Figure 8: Average percent cover of highlighted native dune species over five years in the Grand Sable Dunes, Pictured Rocks National Lakeshore, Grand Marais, MI. The diamond-points and dark lines represent treatments sprayed with Milestone and the lighter square-points and lines represent treatments sprayed with Transline. The light grey box shading years 2011 and 2012 reflects when the treatments were not sprayed with any herbicide.

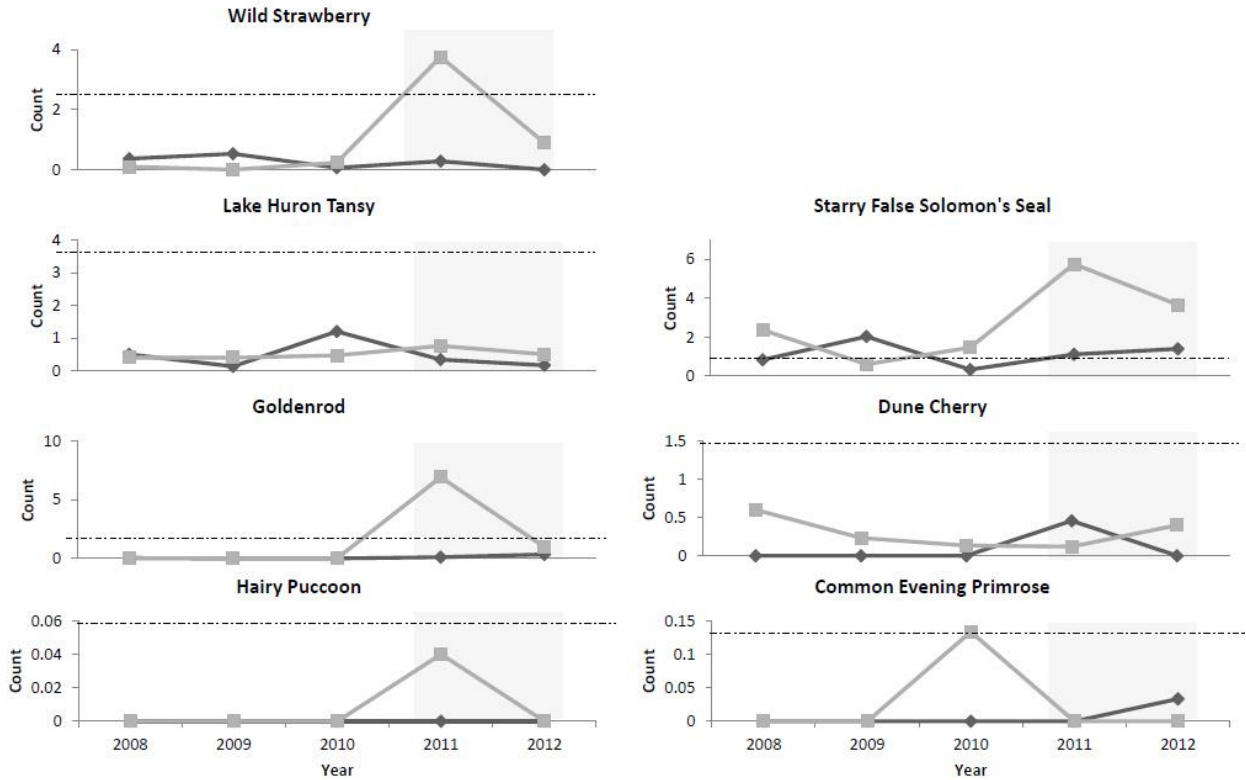


Figure 9: Average number of individuals of highlighted native dune species over five years in the Grand Sable Dunes, Pictured Rocks National Lakeshore, Grand Marais, MI. The diamond-points and dark lines represent treatments sprayed with Milestone and the lighter square-points and lines represent treatments sprayed with Transline. The light grey box shading years 2011 and 2012 reflects when the treatments were not sprayed with any herbicide.