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Melinis repens Seed Bank Longevity in Miami-Dade County

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MELINIS REPENS SEED BANK LONGEVITY IN MIAMI-DADE COUNTY

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Cara Cooper

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To: Dean Kenneth Furton
College of Arts and Sciences

This thesis, written by Cara Cooper and entitled *Melinis repens* seed bank longevity in Miami-Dade county, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this thesis and recommend that it be approved.

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The thesis of Cara Cooper is approved.

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ABSTRACT OF THE THESIS

MELINIS REPENS SEED BANK LONGEVITY IN MIAMI-DADE COUNTY

by

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Professor Hong Liu, Major Professor

The main objective of this research was to determine the seed bank longevity of *Melinis repens* at two Southern Florida sites. Seeds were divided among different exposure levels (shade versus sun) and depths (surface versus buried) and tested for baseline viability using 2,3,5-Triphenyl-tetrazolium chloride. Statistical analysis determined that at the pine rockland site there was a significant interaction between time, exposure, and depth. The initial mean viability at this site declined from 49.71% to 11.26% and 13.06% for sun/buried seeds and sun/surface seeds, respectively, by month 8. The mean viability of shade/surface seeds and shade/buried seeds declined to 24.56% and 22.06% after 8 months. There were no significant effects in the Florida scrub. In order for land managers to completely remove this species from a site, treatment with herbicide will need to continue for a minimum of one year to effectively kill all viable seeds in the seed bank.

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I. INTRODUCTION

Invasive species management is a growing and costly problem for conservation lands. Conservation lands which maintain the basic ecosystem structure and composition have a high conservation value (Bradley 1998). Non-native, invasive species destroy the delicate balance between native species and degrade the conservation value of a natural area (Pimentel *et al*. 2005). Non-native, invasive species are a huge burden on taxpayers in the United States of America and the problem is continuing to grow (Pimentel *et al*. 2005). Invasive species are responsible for adding to human health problems, degrading natural ecosystems, and lowering agricultural production (Lodge et al. 2006). Land managers spend a large amount of time and other resources in the fight against invasion.

The need for protecting ecosystem structure and reducing the burden on American tax-payers is why research is needed to develop management strategies for controlling invasive species, while still allowing native species to thrive in the habitat. A study by Pimentel et al. (2005) found that the United States spends in excess of $120 billion a year to combat non-native species. That amounts to approximately $1000 per household annually in taxpayer monies being spent on invasive control (Melbourne et al. 2007).It is likely that this figure grossly underestimates the true cost of invasive species management because the study only accounts for a handful of the many species under current management (Melbourne et al. 2007). There is a great need to develop management strategies that are effective against invasive species, and affordable in order to curb increasing costs of controlling the spread of exotic species.

A major problem faced by land managers is determining how long monitoring of a target invasive species should continue after initial treatment. Even when a preliminary assessment indicates that a control method has been successful (i.e., no individuals were detected during survey), it is likely that some individuals are overlooked, or new seedlings have yet to emerge (Davis 2009). Land managers must decide how much time and resources should be allocated to follow up monitoring while balancing the risks of re-invasion and wasted funds (Davis 2009). One key component to the successful management of invasive species is understanding the contribution of the seed bank and seed persistence in the control equation.

Monitoring the seed survival rate in the seed bank along with the relative conditions that influence survival rates can be key to the management of invading populations. Information will offer insight into how long emergence from the seed bank will continue to add to the population after the above-ground seed source is removed (Ellsworth et al. 2003). More research into ecosystem-specific and site-specific seed banks is needed, since this type of research can help to predict which plant species will emerge following a disturbance event (Hill and Kloet 2005). Site-specific seed bank research is the most helpful for land managers because it takes into account the target species and the history of the site (Davis 2009).

Melinis repens (Willd.)Zizka is a perennial grass, which is native to South Africa and is known throughout the world to have weedy tendencies (Stevens and Fehmi 2009). It has been previously called both *Rhynchelytrum repens* (Willd.) C. E. Hubbard and *Tricholaena repens* (Willd.) Hitchcock (Stokes 2009). This species readily inhabits disturbed areas and highway medians throughout the state of Florida, and is a serious problem in Southern Florida, where it has begun to disrupt natural areas (Possley and Maschinski 2006). The purpose of my project was to aid in the development of a Best Management Practice Plan for *M. repens*, to be used by the Miami-Dade County land management team. More specifically, my research aims to determine the length of time *M. repens* seed persist in the seed bank in both pine rockland and scrub ecosystems and whether seed bank longevity of *M. repens* varies between sites, or habitat types. The experimental design incorporated the factors of seed depth (surface vs. buried) and sun exposure (sunny vs. shady). Information should aid land managers in determining the proper way to manage the target invasive species and how long treatment will need to continue in order to wipe out the stock of viable seeds in the seed bank.

II. LITERATURE REVIEW

Invasive Species

A non-native, invasive species is defined as species which is threatening or causing harm to habitats or species outside its natural range (Pejchar and Mooney 2009). Invasive species exclude native species from their habitats through ecological processes; such as competition and predation (Boehn et al. 2008). Successful invasive species are able to persist through three phases; introduction, naturalization, and invasion (Richardson et al. 2000). The introduction phase is characterized by the species moving into an area outside of its natural range, usually through the aid of human activity (Richardson et al. 2000). During the naturalization phase the introduced species must overcome the barriers of the introduction, the new environmental conditions, and must be able to reproduce successfully in the new location (Richardson et al. 2000). The final phase is invasion where the species is able to take full advantage of the new habitat and competes with native species for the available resources (Richardson et al. 2000).

Invasive species are considered one of the largest threats to biodiversity worldwide, ranking second to habitat loss/destruction (Wilcove et al. 1998). Invasion can alter the structure and function of an ecosystem as well as the ecosystem services associated with it (Pejchar and Mooney 2009). Invasion tends to be associated with disturbed areas and edges, for example roadsides and agricultural areas, however, some species can successfully invade natural areas or areas with relatively low disturbance (Ellsworth et al. 2003). It is very difficult to eradicate completely an invasive species from a site once it has become well established or spread over an area greater than one hectare (Davis 2009). About half of all federally listed threatened and endangered species in the United States are directly at risk of extinction because of their interactions with non-native, invasive species (Wilcove et al. 1998).

In some cases eradication is not feasible and land managers must choose to control select populations, try to mitigate the results of the invasion, or accept the changing habitat (Lodge et al. 2006). Target species need to be reduced or eliminated before spread becomes too wide to combat (Lodge et al. 2006). A key element for successful invasive species management is to learn the reproductive habits of the target species to understand how reproduction will be influenced by environmental factors as well as to identify the most effective stages for intervention (Cousens and Mortimer 1995).

The Role of Seed banks in Invasion

The seed bank plays an important role in a habitat because it can be used to predict population emergence and dynamics in a community (Mourik et al. 2005). Plant expansion is usually limited by the availability of viable seeds (Mourik et al. 2005). An invasive species is able to use the seed bank as a mechanism for out-competing native species by building up propagules in the seed bank and delaying germination until an appropriate time (Chesson 1994). Identifying propagule pressure can be an important factor in invasive species management (Davis 2009). Propagule pressure can come from within the site (propagule rain, seed bank) or from outside of the site via dispersal from nearby areas (Davis 2009). Management of propagule sources allows land managers to have a much better chance of exterminating a target species (Davis 2009).

There is a strong link between seed production and seed bank accumulation (Pieterse and Cairns 1988). Species that produce more seeds tend to build up in the seed bank and persist for a longer period of time than species that have lessabundant seeds (Pieterse and Cairns 1988). After dispersal, seeds are removed from the seed bank either through germination, natural death that results from aging, decay (Marchante et al. 2010) or predation (Hulme 1998). Monitoring the seed survival rate in the seed bank, along with the relative conditions influencing the survival rates, can be key to the management of invading populations because it will offer insight into how long seed emergence from the seed bank will continue to add to the population after the seed source is removed (Ellsworth et al. 2003).

For some species seeds may persist in the seed bank for many years, and act as a source of propagules even after all above-ground plants have been removed (Cavers and Benoit 1989). It is likely that that seed persistence is associated with environmental conditions, such as soil type and microhabitat (Hill and Kloet 2005, Liu and Pemberton 2008). Seeds persisting in the seed bank may germinate on the basis of different signals such as moisture, temperature, or after an after-ripening event (Cochard and Jackes 2005). Invasive species that are left untreated are more likely to build up in the seed bank and will require longer treatment in order to eradicate the entire seed supply (Marchante et al. 2010).

In order for land managers to triumph over invasive species with persistent seeds, seed bank stores need to be reduced (Richardson and Kluge 2008). As a result there is a need for more background knowledge of seed banks and species seed biology (Plucknett et al. 1977). Unfortunately, seed bank exploration can be very burdensome, both in labor and cost and since there are few replicate studies available for specific ecosystem seed banks results are often difficult to interpret (Thompson et al. 1993). By studying these seed banks within affected ecosystems, land managers will receive information that is directly applicable to their work (Davis 2009).

Melinis repens impact in Miami-Dade County

Melinis repens was brought into the United States around 1866 for ornamental purposes and introduced as a forage plant a decade later in trial gardens for the U.S Department of Agriculture (Tracy 1916, Mislevy and Quesenberry 1999). It was reported to begin spreading soon after introduction (Tracy 1916). By the year 1916, over 40,000 acres of *M. repens* hay were in cultivation in Florida (Stokes 2009). It was not long after this that ranchers realized that cattle preferred to eat other species and the Department of Agriculture stopped encouraging its use for livestock (Stokes 2009). Presently, within the United States, the species can be found invading several states. In Florida it does particularly well due to the mild climate and can commonly be found along roadsides and disturbed areas throughout the state (Stokes 2009). It poses a greater threat in South Florida, where it has been found to infest natural communities (Gordon et al. 2005). It has been recognized as a Type I invasive species (threat to native species) by the Florida Exotic Pest Plant Council since 2005 (FLEPPC 2005).

III. Materials and Methods

Study Species

Melinis repens sets seeds two-weeks after flowering begins, however, seeds are much more likely to germinate following an after-ripening period, rather than immediately following dispersal (Stokes 2009). The seeds often accumulate upon the soil surface, forming a layer as deep as several inches (Stokes 2009). *Melinis repens* is very difficult to manage and even if it is successfully eradicated it may just open up areas for other highly invasive species may rapidly replace it (Stokes 2009). It is readily controlled through tillage, and it is almost never a problem in agricultural settings, however, tillage in natural areas is highly undesirable since it would further disturb the substrate (Stokes 2009). There has been little research done on the management of *M. repens* invasion and land managers have become frustrated with traditional methods, such as prescribed fires and manual control, because they do not work well with this particular species (Possley and Maschinski 2006). The suggested form of treatment is to remove any nearby potential seed sources, and then treat the infestation prior to seed set with herbicide, either glyphosate (the most effective chemical) or imazapyr (recommended for selective treatment when desirable native species are intermixed) (Stokes 2009).

Study Sites

Two sites were chosen for study in my project. The first site is the ninth unit at Larry and Penny Thompson Park in southern Miami-Dade County. This parcel is located near the intersection of Southwest 122nd Avenue and Southwest 172nd Street. Larry and Penny Thompson park is a remaining fragment of an extremely rare habitat-type, Pine Rockland (FNAI 2010). The second site was chosen at the northern end of Miami-Dade county. County-Line Scrub is an approximately 15 acre park and natural area located at San Simeon Way and Northeast 215 Street. As its name suggests this Florida Scrub parcel is situated on the county line between Miami-Dade and Broward Counties. These sites were chosen because they are the main upland habitat types which are being conserved and managed by the EEL Program and they are both known to have infestations of the target species.

Field Methods

Experiment 1: Monthly Fresh Seed Viability

I randomly collected *Melinis repens* inflorescences across one of the study sites, Larry and Penny Thompson Park, each month. A minimum of 300 seed dispersal units were then dissected to determine the seed presence. Seeds were subjected to a chemical viability assessment using a 1% solution of 2,3,5-Triphenyl-tetrazolium chloride to stain the viable seed embryos. After the staining period the seeds were examined under a dissecting microscope to determine the percentage of seeds stained.

Experiment 2: Seed Bank Longevity

This experiment was conducted at two sites in Miami-Dade County: one scrub habitat (County-Line Scrub) and one in a pine rockland habitat (Larry and Penny Thompson Park). At each site five full sun plots and five shaded plots chosen at random. Full sun plots had no canopy cover, and shaded plots had at least fifty percent canopy cover. Each plot was be marked by a central piece of rebar and had ten seed bags attached to the central pole with wire. Five of these seed bags were placed on the surface and secured with 1-3 nails and five were buried approximately 2 cm under site-specific soil.

Seed bags were created by cutting 4”x8” strips of Phifer Super Solar Screen and creating the bags with hot glue. Three sides were sealed shut, and 50 seeds and 1-2 teaspoons of sterile sand were placed inside each bag. The bags were then glued closed. A pair of bags (one surface and one buried) were collected from each plot after 1 month, 3 months, and 8 months and brought back to the lab for viability testing. The seeds were removed from the bags, examined and counted before being subjected to a chemical viability test using a 1% solution of 2,3,5-Triphenyl-tetrazolium chloride.

Laboratory Methods

The method used for chemically staining the seeds was adapted from the Tetrazolium Testing Handbook for Agricultural Seeds (Association for Official Seed Analysts 1970). This test allows for viability to be measured using a stain. Tetrazolium is taken up into the cells of the embryo and reacts if cell metabolism is detected. Viable seeds are stained pinkish, red. A mixture of 1% Triphenyl tetrazolium chloride solution was made by adding 1 gram of the chemical to 100 milliliters of distilled water. Seeds were manually removed from their dispersal units and placed in to a small petri dish. Solution was then added to completely submerge the seeds. The petri dishes were then cover and left in a warm, dark place (a drawer) to incubate for 6-12 hours (until development was visible). A dissecting microscope was used to visually determine viability based on embryonic staining.

Statistical Analysis:

<u>Experiment 2:</u>

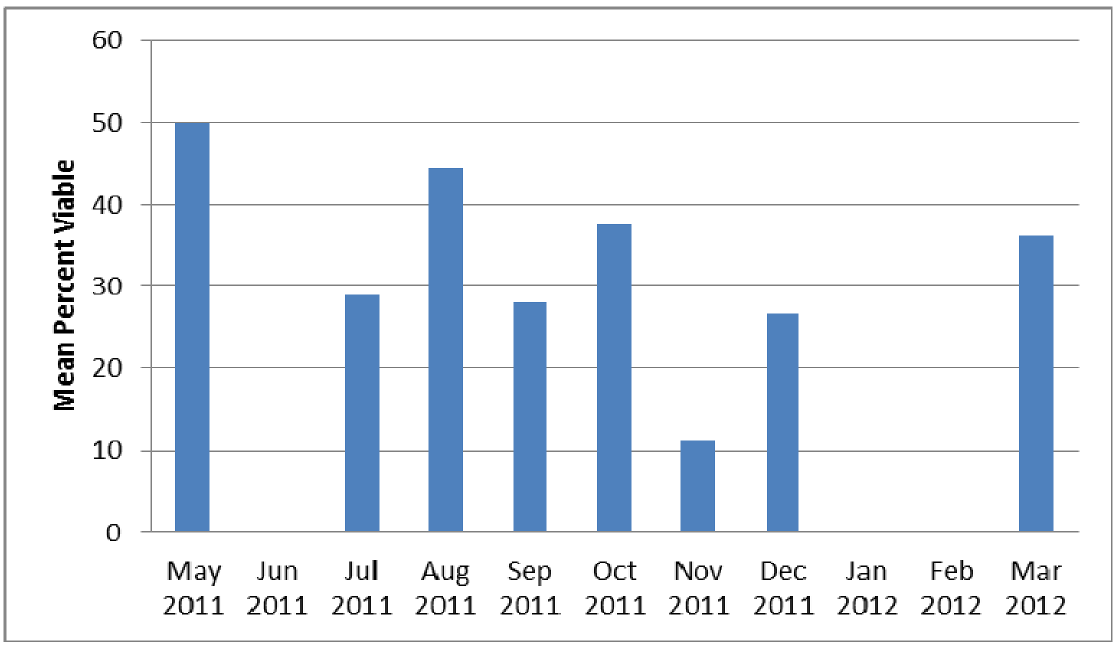
A Three-Way ANOVA test was used to determine the impacts of habitat type (pine rockland vs scrub, exposure (sun vs. shade) and burial (buried vs. surface) treatments on the percentage of seeds viable at each time interval. A Repeated Measures ANOVA test was also used to determine the strength of the data across different tests. The significant terms were then subjected to the Holm's Sequential Bonferroni Correction Method to determine which sets of pairwise variables were significantly different.

IV. RESULTS

Experiment 1; Fresh Seed Monthly Viability

The fresh seeds collected from the monthly sampling at Larry and Penny Thompson Park, Unit 9 pine rockland indicated an overall decreasing trend in percent seed viability from early summer (May 2011) to early spring (March 2012). (see figure 1). Sampling was not conducted in January and February 2012 because of low seed availability.

Figure 1. Mean percentage of viable seeds on a month-month basis in a pine rockland site from May 2011 to March 2012 in Southern Florida.



Experiment 2

Melinis repens seeds behaved very differently in the Pine Rockland habitat than in the Scrub habitat. Pine Rockland seeds seemed to have a much higher initial viability, 49.7% compared to the Scrub site, 14.0% (table 1). While there appeared to be a drop in mean viability over time in the Scrub habitat the change was not statistically significant (F(3,65)=0.932, P=0.430). There was no significant difference in viability between time periods, exposure, or depth in the Scrub (table 2 and figure 2). The pine rockland site showed a significant three-way interaction among the factors, ie. time, exposure(sun vs shade), and depth (surface vs buried) (F(3,65)= 4.049, P=0.011)(table 3). The pine rockland site also showed an overall decrease in viability over time (figure 3). Seeds placed in the sun (either surface or buried) showed a significant difference in viability between the baseline and 3 months (P=0.0001) and 8 months (P=0.0001) as well as a significant difference between month 1 and 3 months (P=0.0001) and 8 months (P=0.0001) (table 4). Seeds placed in the shade and on the surface also behaved in a similar manner, with a significant difference between the baseline (month 0) and months 3 (P=0.0001) and 8 (P=0.001) and a significant difference between month 1 and months 3 (P=0.000) and 8(P=0.001) (table 4). In these three treatment combinations the major decrease in seed viability happened between month 1 and month 3 (figure 4). However, seeds that were buried in the shade behaved differently. In these seeds viability remained fairly constant and the only significant difference was between the baseline (month 0) and month 8 (table 4 and figure 4). These results were consistent when using the Repeated Measures analysis

Table 1. Seed Viability estimates of *Melinis repens* for the time variable moderated by exposure and depth at a pine rockland site and a scrub site in Southern Florid

Site	**Time Since Buried (months)**	**Mean**	**Standard Error**
Scrub	0	14.002	2.137
	1	12.954	2.341
	3	13.272	2.341
	8	9.057	2.341

Pine Rockland	0	49.709	2.370
	1	44.929	2.475
	3	14.657	2.475
	8	17.734	2.551

Table 2. Three-Way ANOVA table showing the effects of all three treatments and their interactions on the mean percent viable seeds of *Melinis repens* at a scrub site in Southern Florida.

Source	**Type III Sum of Squares**	**Degrees of Freedom**	**Mean Square**	**F**	**Sig.**	**Partial Eta squared**
Corrected Model	1577.703	15	105.180	0.960	0.505	0.175
Intercept	12672.689	1	12672.689	115.667	0.000	0.630
Sun_Shade	290.555	1	290.555	2.652	0.108	0.038
Buried_Surface	27.703	1	27.703	0.253	0.617	0.004
Time	306.423	3	102.141	0.932	0.430	0.040
Sun_Shade x Buried_Surface	150.839	1	150.839	1.377	0.245	0.020
Sun_Shade x Time	714.006	3	238.002	2.172	0.099	0.087
Buried_Surface x Time	64.561	3	21.523	0.196	0.898	0.009
Sun_Shade x Buried_Surface xTime	64.561	3	21.520	0.196	0.898	0.009
Error	7450.231	68	109.562			
Total	21946.018	84				
Corrected Total	9027.935	83				

Figure 2. Bar graph comparing the mean percent viable seeds of *Melinis repens* of each treatment over time, since project initiation, at the Scrub site. Error bars represent standard error.

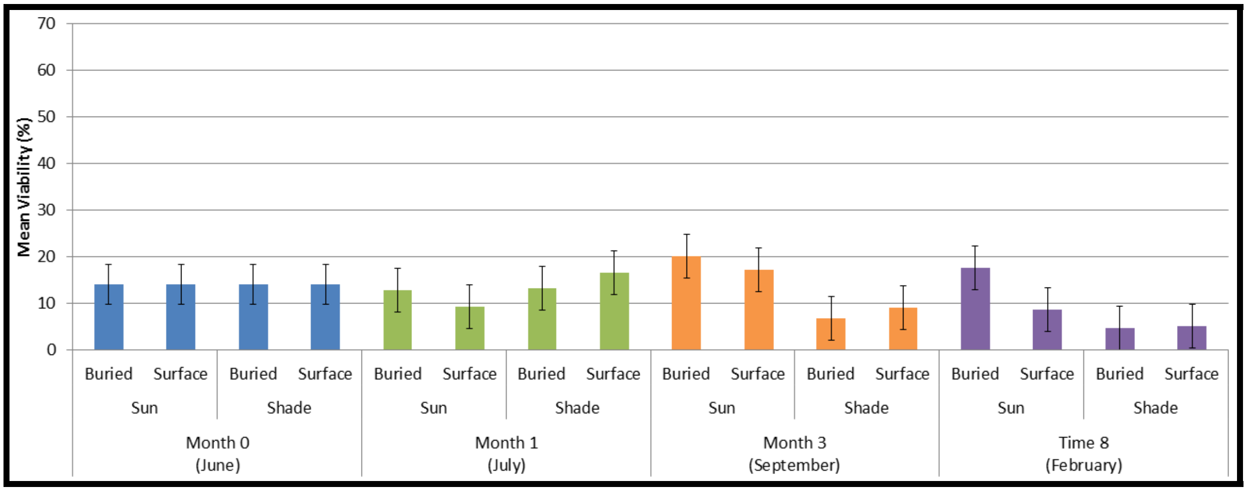


Table 3. Three-Way ANOVA table showing the effects of all three treatments and interactions on the mean percent viable seeds at a pine rockland site in Southern Florida.

Source	Type III Sum of Squares	Degrees of Freedom	Mean Square	F	Sig.	Partial Eta squared
Corrected Model	24206.56	15	1613.771	13.170	0.000	0.752
Intercept	82092.58	1	81091.58	661.766	0.000	0.911
Sun_Shade	684.113	1	684.113	5.583	0.021	0.079
Buried_Surface	69.792	1	69.792	0.570	0.453	0.009
Time	20038.87	3	6676.291	54.483	0.000	0.715
Sun_Shade x Buried_Surface	55.334	1	55.334	0.452	0.504	0.007
Sun_Shade xTime	1147.269	3	382.423	3.121	0.032	0.126
Buried_Surface	486.125	3	162.042	1.322	0.275	0.058

x Time						
Sun_Shade x Buried_Surface xTime	1488.639	3	469.213	4.049	0.011	0.157
Error	7964.980	65	122.538			
Total	116629.5	81				
Corrected Total	32171.54	80				

Figure 3. Bar graph comparing the mean percent viable seeds of each treatment over time, since project initiation, at the Pine Rockland site. Error bars represent standard error.

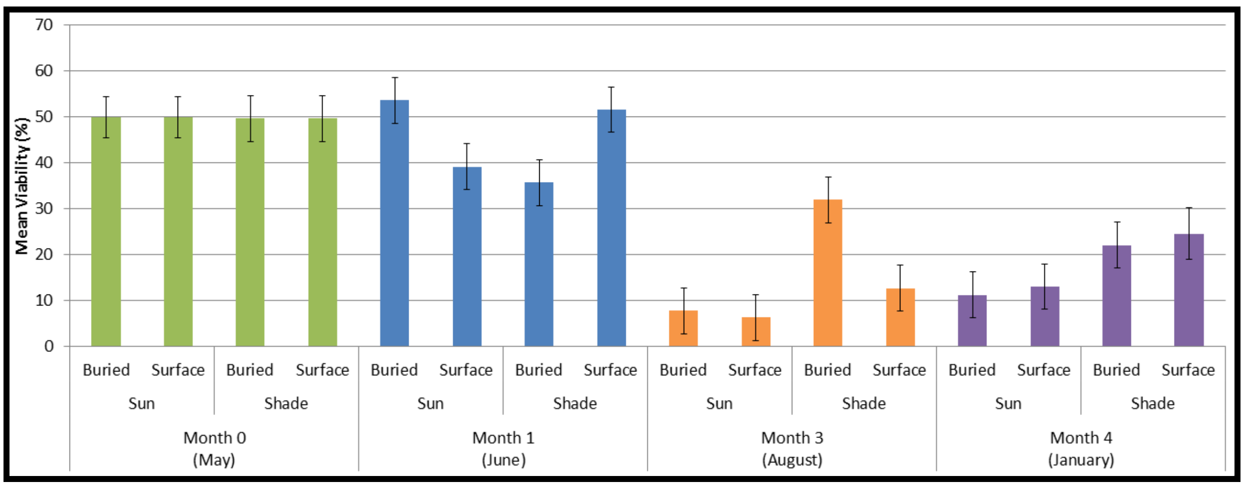
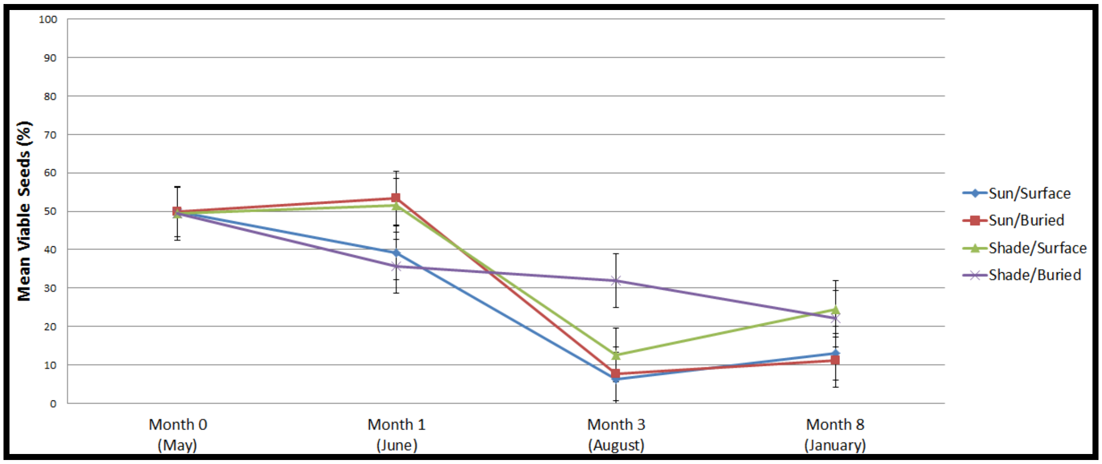


Table 4. Pairwise comparison of seed viability of *Melinis repens* showing the differences among treatments at different times at a pine rockland site in Southern Florida.

Exposure	Depth	(I) Time	(J)Time	Mean Difference (I-J)	Std. Error	Sig.
Sun	Buried	1	2	-3.653	6.703	0.588
			3	42.110	6.703	0.000
			4	38.608	6.703	0.000
		2	1	3.653	6.703	0.588
			3	45.763	7.001	0.000
			4	42.260	7.001	0.000
		3	1	-42.110	6.073	0.000
			2	-45.763	7.001	0.000
			4	-3.502	7.001	0.619
		4	1	-38.608	6.703	0.000
			2	-42.260	7.001	0.000
			3	3.502	7.001	0.619
Sun	Surface	1	2	10.787	6.703	0.112
			3	43.561	6.703	0.000
			4	36.806	6.703	0.000
		2	1	-10.787	6.703	0.112
			3	32.774	7.001	0.000
			4	26.018	7.001	0.000
		3	1	-43.561	6.703	0.000
			2	-32.774	7.001	0.000
			4	-6.755	7.001	0.338
		4	1	-36.806	6.703	0.000

Exposure	Depth	(I) Time	(J)Time	Mean Difference (I-J)	Std. Error	Sig.
		4	2	-26.018	7.001	0.000
			3	6.755	7.001	0.338
Shade	Buried	1	2	13.930	7.001	0.051
			3	17.691	7.001	0.014
			4	27.497	7.001	0.000
		2	1	-13.930	7.001	0.051
			3	3.761	7.001	0.593
			4	13.568	7.001	0.057
		3	1	-17.691	7.001	0.014
			2	-3.761	7.001	0.593
			4	9.806	7.001	0.166
		4	1	-27.497	7.001	0.000
			2	-13.568	7.001	0.057
			3	-9.806	7.001	0.166
Shade	Surface	1	2	-1.944	7.001	0.782
			3	36.889	7.001	0.000
			4	24.990	7.426	0.001
		2	1	1.944	7.001	0.782
			3	38.832	7.001	0.000
			4	26.934	7.426	0.001
		3	1	-36.889	7.001	0.000
			2	-38.832	7.001	0.000
			4	011.899	7.426	0.114
		4	1	-24.990	7.426	0.001
			2	-26.934	7.426	0.001
			3	11.899	7.426	0.114

Figure 4. Differences in seed viability of *Melinis repens* at a pine rockland site in Southern Florida between the shade/buried treatment at time 3 compared to the sun/surface, sun/buried, and shade/surface treatments.



V. DISCUSSION

Field Observations

After observing populations of *Melinis repens* at a pine rockland site and a scrub site in Southern Florida some obvious patterns emerged. It appeared that this plant species continually sets seed throughout the year, with a reduction in seed-set and availability during the dry winter months (January-February). These months also correlated with lower viability levels of the available seeds at the surveyed pine rockland site. Unfortunately, the reduction in seed production is only temporary, and when the warmer, wetter months begin the species bounced back. This is consistent with reportings that *M. repens* can be stunted by cold weather, but requires a hard freeze to cause plant mortality (Stokes 2009). At both sites the extent of invasion seemed to stay fairly consistent throughout the year other than the temporary winter die-back. I never observed a plant which was affected by either pest or disease.

Overall, the pine rockland site appeared to be more impacted by the invasion, than did the scrub, because *M. repens* forms a thick stand that did not include other species. At the scrub site the infestation appeared to occur in smaller patches (1-2 meters), with other species able to share the available space with the exotic species.

Seed Bank Longevity of *Melinis repens*

At the Larry and Penny Thompson Park pine rockland site, initial viability was close to 50% and remained at that level for the first month (June) in the seedbank. Viability of seeds placed in the sun (at either depth), or in the shade and on the surface decreased to around 10% by the third month (August) and stayed near that level through the eighth month(January). Seeds placed in the shade and buried, however, seemed to retain their viability longer than those in full-sun or on the surface in the shade, with a significant drop not occurring until the eighth month. Even at the eighth month seeds that were shaded and buried still had a mean viability of around 22% (nearly double that of their surface and sunny counterparts).

There are several factors which may result from being shaded and/or buried that could increase seed persistence in the seed bank. One contributor to the loss of viable seeds in the seed bank is germination. Germination can be triggered by a number of different environmental processes, including temperature and exposure to light (Baskin and Baskin 1998). These same processes are also known to cause seeds to age (Walters 1998). Seeds which are dependent on light cues for germination will be more likely to remain viable in the seed bank when dispersed into areas that are shaded. It is biologically more advantageous to wait for a break to form in the canopy that would allow for increased sunlight before germinating. Temperature is also a factor which can contribute to both germination increases and seed mortality (Lonsdale 1993). The shade offers a buffer against temperature fluctuations, which can signal germination to begin, and against extreme high temperatures which may cause seed death. A study of the invasive vine species *Paederia foetida* in Florida ecosystems, illustrated that seeds which were placed in the shaded, interior forest habitats, retained viability longer than compared to sunnier, open canopy habitats (Liu and Pemberton 2008). This is especially important to note with the knowledge that global climate change will be causing increasing soil temperatures (Ooi et al 2009). However, shading can also have some detrimental effects on seed longevity as well. Many studies have shown that shaded microclimates retain moisture longer than open canopy sites and this promotes soil pathogen and fungi growth (Augspurger and Kelly 1984, Forget 1997, O'Hanlon and Kotanen 2004). Seeds which are in shaded seed banks are more likely to be subjected to a fungal attack than seeds which are not shaded (O'Hanlon and Kotanen 2004).

Seed burial is also known to increase seed longevity in the seed bank (Lonsdale et al. 1988). Burial can offer some of the same protection from germination triggering environmental cues and seed mortality risks as shade, including limiting light exposure and buffering temperature fluctuations as well as other protections from environmental hazards (Facelli et al. 2005). Burial is actually more effective than shade alone at blocking light and temperature from affecting seeds. The further away from the surface a seed is buried, the less it is exposed to diurnal temperature fluctuations (Pierson and Wright 1991).One study, looking at buried versus surface seeds of *Artemisia tridentata* (Nutt.) found that seeds which were buried retained three times higher viability than those which were placed on the surface (Wijayratne and Pyke 2012). Unlike seeds which may be located in a shaded, surface position in the seed bank, seeds which are buried may be less likely to succumb to a fungal infection. For example, in the case of *A. tridentata,* buried seeds were 25% less likely to be lost as a result of fungal infection than surface seeds (Wijayratne and Pyke 2012). Buried seeds are also more protected from seed predation, according to a study looking at seed predation in deciduous woodlands, seeds which were buried at a depth of 1 cm were 50% less likely to be encountered by a predator than surface seeds (Hulme and Borelli 1999).

At County-Line Scrub the initial seed viability was much lower than that of the pine rockland site. This may explain why there is a more intense invasion occurring in the pine rockland. Grasslands of South Africa (*M. repens*' native habitat) tend to have soils which are slightly acidic with a calcareous underpan, and high levels of organic matter (Palmer and Ainslie 2005). These grasslands also receive a relatively high amount of precipitation and are subject to seasonal flooding (Palmer and Ainslie 2005). These conditions closely mimic the traits of pine rockland ecosystems, but not those of scrub ecosystems (FNAI 2010). Scrub habitats tend to be low in available nutrients and organic matter, and have drier, quickly draining, sandy soils (FNAI 2010). Since no monthly viability survey was conducted at the scrub site, there are no data to show whether or not the initial viability was consistently low throughout the year, or if the month that the baseline seeds were collected (June 2011) just happened to have a particularly low mean viability.

Even though there were no statistically significant differences in the mean viability at month 0 and month 8 at the scrub site, there does seem to be a slight downward trend. It is likely that the reason that there was no significance was because of extremely high variability between the replicates. There was also no difference in seeds which were buried in the shade from the seeds in the sun and/or on the surface at this site. For some reason, the soil did not offer the same benefits in the scrub compared to the pine rockland site. One possible explanation for this phenomenon is that scrub sites have sandy soils which do not retain moisture and have little organic matter to insulate the top of the soil. Perhaps this lack of organic matter and moisture negates the ability of the soil to act as a buffer for temperature fluctuation.

On the basis the results of this study it is likely that *M. repens* has a short-term persistence of at least one year in the seed bank in ecosystems, but further research is needed to determine the full longevity of this species in the seed bank. According to a study conducted in Western Europe most invasive species have a transient (less than one year) to short-term persistence in the soil seed bank (Thompson et al. 1995). There is one remaining set of seed bags at both sites, with a retrieval date of one year. The last set of seed bags should provide more insight on total longevity, but a longer study is needed. More information is also needed on the soil composition, precipitation, and available light at these sites to adequately determine which factors are responsible for variations among treatments. I would also suggest a laboratory study which mimics this study, without the use of mesh seed bags to compare to the field results because several studies have reported potential problems associated with seed bags (Mourik et al. 2005, Wijayratne and Pyke 2012). These studies show that mesh seed bags may affect the microenvironment of the seeds and this may either result in an overestimation of viability, if the bag prevents the seeds from coming in contact with soil organisms, which normally could destroy the seeds, or an underestimation of total longevity, because of the promotion of mold and fungi without interaction with the soil (Mourik et al. 2005, Wijayratne and Pyke 2012).

VI. CONCLUSION

The current ecological situation at both sites is unfortunate. *Melinis repens* and other non-native species are invading and out-competing the native species that would normally call these ecosystems home (Possley and Maschinski 2006). This is especially true at the pine rockland site, where the tall stalks of *M. repens* have formed thick stands and very little understory diversity is visible and is further complicated by the fact that it is now known that seeds of this species can persist for at least eight months in the soil and on the soil surface. Add in the fact that global climate change threatens to change habitats, global temperatures, and precipitation patterns and land managers have quite an unpredictable challenge on their hands. While higher soil temperatures from an increasingly warm world could be beneficial in speeding up the death of invasive seeds in the seed bank, this effect would be equally detrimental to the seeds of native species stored in the soil as well.

Due to the sheer volume of individual plants and the area of land that they occupy, the most effective treatment would be with a broadcast herbicide to initially kill the adult population. This should then be followed by a spot treatment herbicide to target seedlings which emerge from the seed bank. Since both habitats are home to native species, including rare or endangered plants and animals, care should be taken to use an herbicide which is tolerated by the native species (such as imazapic) and to apply herbicides with the lowest dose necessary to complete the job. Follow up treatments will need to continue for a minimum of one year, and perhaps longer if new seeds are introduced into the system through wind dispersal from nearby infestations. However, herbicide applications can be costly, and in the case of this particular pine rockland site, it may be in the best interest of land managers to focus their efforts on preventing *M. repens* from spreading to adjacent natural areas and forming thick stands (ie. focusing treatment on the edges), rather than the ambitious goal of removing the species all together and restoring the infested area.

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