



Project Title: Impacts of exotic phreatophyte management on the invasiveness of perennial pepperweed and Russian knapweed.

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1.) PROJECT ACCOMPLISHMENTS:

OBJECTIVE #1: Determine the ranges of light levels that exist under canopies of large stands of phreatophytes that are currently being managed.

Canopy architecture and light levels were characterized for canopies of the invasive exotic, tamarisk (*Tamarix ramosissima*), and in the native cottonwood (*Populus deltoids*). Four sites were selected along the Middle Rio Grande River where restoration projects were underway to remove tamarisk and eventually restore them to native cottonwood riparian habitat (bosque) including two National Wildlife Refuges (NWR) (Table 1 and Figure 1). The sites were selected because they were in various stages of restoration and they possessed overlapping assemblages of invasive species including the two under-story species with which we were interested: perennial pepperweed (*Lepidium latifolium*) and Russian knapweed (*Acroptilon repens*).

TABLE 1. Study sites assessed and species present.

Location	Species present
Bosque Del Apache NWR (BDA)	tamarisk
Albuquerque Open Space (ALB)	tamarisk, cottonwood, knapweed, pepperweed
San Antonio Restoration Area, Socorro (SA)	cottonwood, knapweed
Sevilleta NWR (SNWR)	tamarisk, pepperweed

Between 20 and 50 plants at each site were measured for leaf area index (LAI, m^2 leaf m^{-2} ground) and photosynthetic photon flux ($\mu mol\ m^{-2}\ s^{-1}$). A plant canopy analyzer was used to estimate LAI which utilizes the canopy gap fraction method (Licor Biosciences, LAI-2000, Lincoln, NE). Photosynthetic photon flux (PPF) was estimated using a ceptometer with

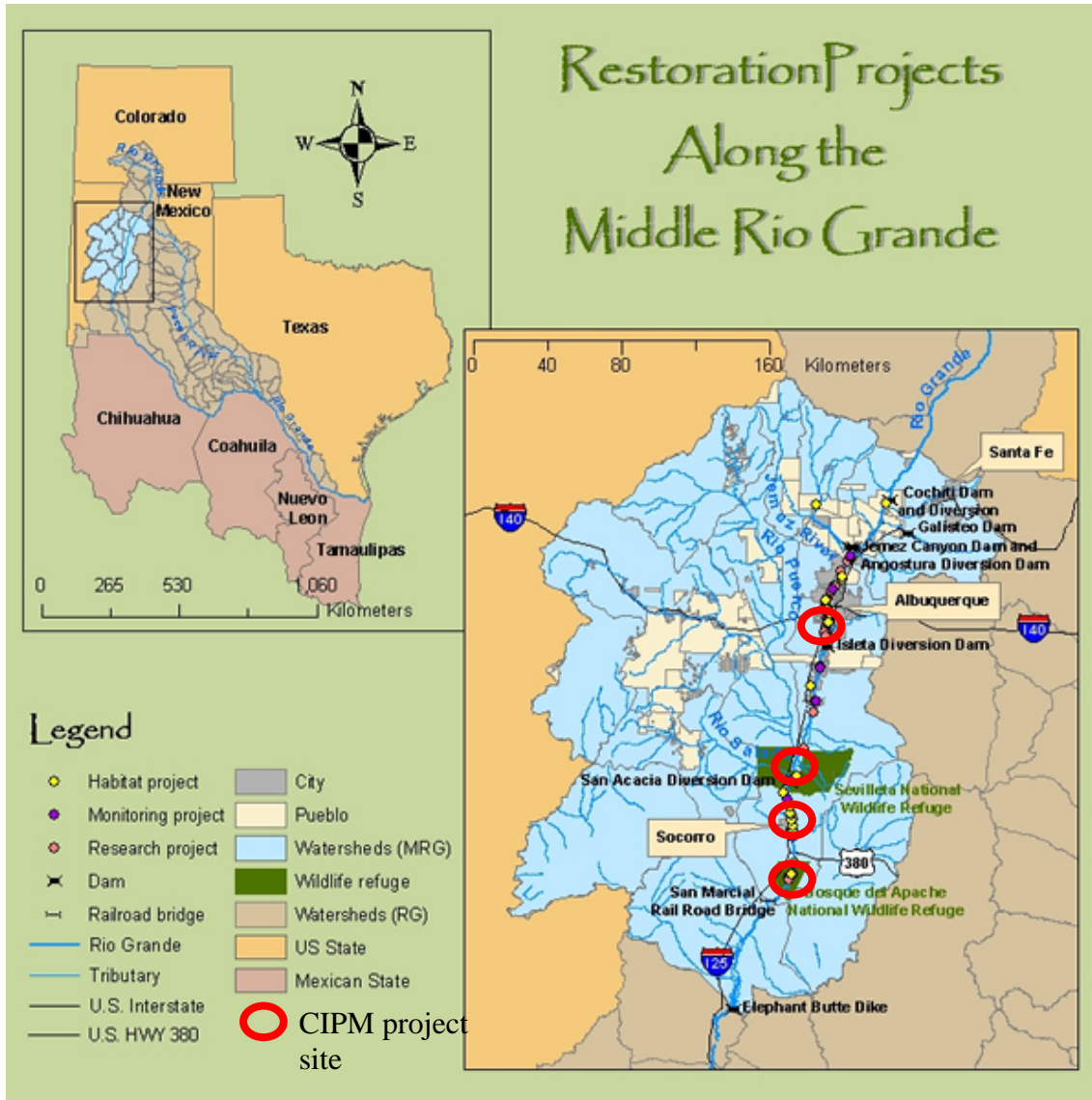


FIGURE 1. The four sites selected for this study along the middle Rio Grande river as listed in Table 1.

Source: http://www.fws.gov/southwest/mrgbi/maps/Rio_Grande_Basin/index.html

integrated probe and microcontroller (Decagon

Devices Inc., AccuPAR model LP-80, Pullman, WA). Sufficient readings were collected with the ceptometer and the canopy analyzer at each site to accurately represent the range of light levels and leaf canopy architecture at each site. All sites were surveyed in 2005 and 2006 to characterize the light conditions and canopy architecture but also to observe changes in canopies as a result of various forms of management.

LEAF AREA INDEX AND PHOTOSYNTHETIC PHOTON FLUX IN UNDER-STORY

The leaf area indices (m^2 leaf m^{-2} ground) at ground level under typical saltcedar and cottonwood canopies were $4.71 (\pm 0.66)$ and $1.45 (\pm 0.20)$, respectively, averaged over all sites that had the species. When light levels were expressed as quadratic functions of height, tamarisk canopies were convex in shape while cottonwood canopies were concave (Figure 2). The positive quadratic coefficient for cottonwood ($c=0.002\pm 0.001$) indicates the foliage was distributed high in the tree with a nearly branchless and leafless bottom portion of the trunk. The negative quadratic coefficient for tamarisk ($c=-0.004\pm 0.002$) indicates that the foliage was distributed primarily at the bottom of the canopy near the ground.

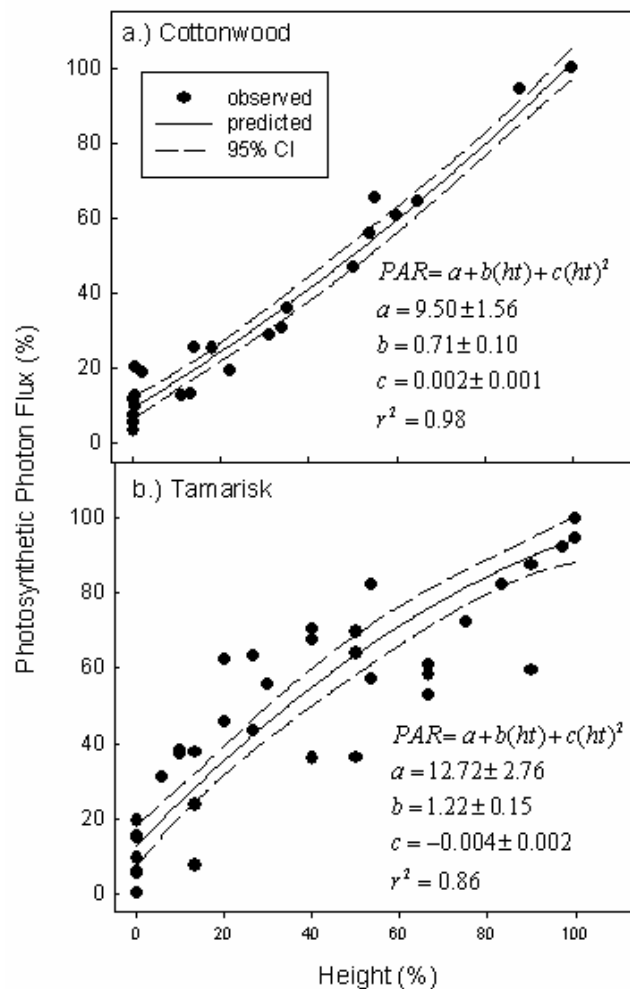


FIGURE 2. Light attenuation from the top (100% height) to the bottom (0% height) of a cottonwood (a) and tamarisk (b) canopy. Quadratic functions and 95% confidence intervals were fit to the observed data

Light flux (PPF) was expressed as an exponential decay function of leaf area index as described by Beer's Law where leaves in the plant canopy were assumed to approximate a turbid medium by using the equation:

$$PPF = a \exp^{-k(LAI)} \quad \text{eq. 1}$$

where PPF is the percent of full sun at the top of the canopy, a is the fitted full sun percentage (should be close to 100%), and k is an extinction coefficient that measures the efficiency at which light is intercepted by the canopy. When expressed this way, the perennial pepperweed canopy possessed a significantly higher extinction coefficient (0.88 ± 0.04) than Russian knapweed (0.43 ± 0.02) (Figure 3). Thus, any increase in tamarisk leaf area over a given area of ground results in a much greater attenuation of light than that same increase for cottonwood leaf area. The differences in extinction between the two species are due to differences in leaf orientation such as leaf angle.

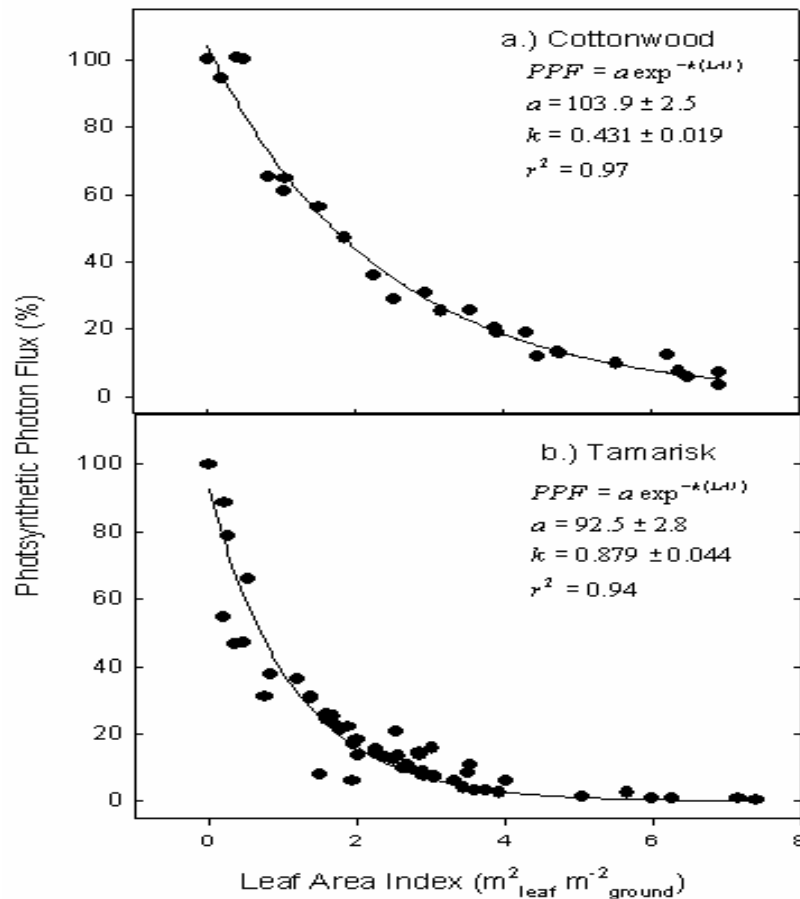


FIGURE 3. Light attenuation as a function of leaf area index of a cottonwood (a) and tamarisk (b) canopy. Exponential decay functions were fit to the observed data to estimate extinction coefficients (k).

OBJECTIVE #2: Survey various populations of perennial pepperweed and Russian knapweed underneath canopies of phreatophytes and measure the light and canopy conditions where they survive.

Light levels and leaf area indices were measured underneath tamarisk and cottonwood canopies at the three sites where the under-story invasive species, perennial pepperweed and Russian knapweed, had infested (see Table 1). The methodology utilized to meet objective 1 was also used to address objective 2. When tamarisk is not controlled in any way, leaf area index reached an average $5.22 (\pm 0.11) \text{ m}_{\text{leaf}}^2 \text{ m}_{\text{ground}}^{-2}$ with a concomitant PPF of $34.3 \pm 1.9 \mu\text{mol m}^{-2} \text{ s}^{-1}$ averaged over all sites (Table 2). The light levels just above the canopies for perennial pepperweed and Russian knapweed probably were well above and below those levels under uncontrolled tamarisk canopies, respectively (PPW/RKW, Table 2). Therefore, light conditions under uncontrolled tamarisk probably would not be conducive for pepperweed or knapweed establishment.

MANAGEMENT AND RESTORATION EFFECTS ON UNDER STORY LIGHT LEVELS

Control measures included mowing at the ALB site and burning at the BDA site. Burning appeared to remove almost all of the foliage of tamarisk (Table 2). However, unless light levels were subsequently suppressed in some way following burning, the under-story invasives will have little obstruction to their establishment. Restoration efforts at the ALB site have removed tamarisk and replanted to cottonwood. Unfortunately, PPF levels under these restored cottonwoods were not significantly different from those where pepperweed and knapweed grew (Table 2). Therefore, it appears that greater light suppression or additional control measures for the under-story invasives will likely be required with restoration efforts where under-story invasives are present.

TABLE 2. Average photosynthetic photon flux (PPF \pm SE, $\mu\text{mol m}^{-2} \text{ s}^{-1}$) and leaf area index (LAI \pm SE, $\text{m}_{\text{leaf}}^2 \text{ m}_{\text{ground}}^{-2}$) across all sites at ground level at the top of canopies of perennial pepperweed and Russian knapweed (PPW/RNW), where tamarisk was not controlled (no control), where it was mowed, where it was burned, and where it was removed and replanted to cottonwood (restored).

MANAGEMENT	PPF	LAI
no control	34.3 ± 1.9	5.22 ± 0.11
PPW/RKW	391.3 ± 144.4	2.59 ± 0.45
burned	957.5 ± 9.8	0.02 ± 0.04
mowed	211.7 ± 9.0	2.25 ± 0.08
restored	319.9 ± 11.4	1.67 ± 0.06

OBJECTIVE #3: Assess perennial pepperweed & Russian knapweed growth response to the range of light conditions that exist under exotic phreatophyte canopies.

PHOTOSYNTHESIS

Photosynthesis was measured on at least four leaves from twenty randomly selected tamarisk, Russian knapweed, and perennial pepperweed plants that were grown in open and unshaded conditions at the San Antonio Restoration Area (SA) and at the Albuquerque Open Space (ALB). Measurements were collected under field conditions using a Licor 6400

Photosynthesis System which measures CO₂ and moisture flux to estimate net carbon assimilation and transpiration (Licor Biosciences, Inc., Lincoln, NE). Additional measurements were collected from a random sample of 250 perennial pepperweed plants that were established from crown-root fragments in 1 and 5 gallon pots using sterilized potting media at California Polytechnic State University in San Luis Obispo during the summer and fall of 2006. These pots were randomly divided into three shade conditions representative of the light conditions that are experienced by understory plants along the Middle Rio Grande river riparian habitat: unshaded, partially shaded (about 50% shade), and shaded (about 80% shade). The 50% shade was established with nursery trays that reduced light by about 50% and the 80% level was established with 50% woven shade cloth doubled over. Light levels were determined with gallium-arsenide-phosphide photodiodes (part # G1118, Hamamatsu Corporation, Middlesex, NJ) circuited and constructed by Steinmaus and read on a Campbell Scientific 23X datalogger.

Sufficient data were collected to develop light curves for tamarisk, Russian knapweed, and perennial pepperweed. The light gradient was generated in the field by tilting chamber to a sufficient angle relative to the sun so that the quantum sensor read to proper light level. Light gradients in lab were generated with controlled LED lights using the GaArP sensors to validate PPF. Photosynthetic light response was fitted to a rectangular hyperbolic function which is the same equation used to describe **Michalis – Menten** kinetics:

$$photosynthesis = R + \frac{P_{max}(PPF)}{K + PPF} \quad \text{eq. 2}$$

where R is the estimated dark respiration rate, P_{max} is the estimated light saturated photosynthetic capacity, and K is analogous to the Michalis-Menten constant describing the light level at half the P_{max}. Quantum efficiency (μmol CO₂ fixed per μmol photon captured) can be estimated by K/P_{max}. All parameters were fitted to light response observations for tamarisk, Russian knapweed, and perennial pepperweed leaves that were grown under unshaded conditions (sun) or under shaded conditions (80% shade) (Table 3). Parameter estimates for leaves grown under sunny versus shady conditions

The light compensation points for perennial pepperweed plants grown in the sun and shade were 54.3 and 49.4 μmol m⁻² s⁻¹, respectively, estimated from the fitted parameters from equation 2. For Russian knapweed the estimated light compensation points were 166.2 and 7.3 μmol m⁻² s⁻¹, respectively. Based on the data that was used to generate light response curves of plants grown under shaded and unshaded conditions indicated that perennial pepperweed had a greater ability to acclimate when grown under full sun conditions with a higher light-saturated photosynthetic capacity (22.6 ± 1.48 μmol CO₂ fixed m⁻² s⁻¹) than Russian knapweed (11.6 ± 0.3 μmol CO₂ fixed m⁻² s⁻¹). However, knapweed maintained 81% of its photosynthetic capacity when grown in shaded conditions (9.4 ± 2.3 μmol CO₂ fixed m⁻² s⁻¹) compared to the 62% of P_{max} maintained by pepperweed (14.1 ± 0.3 μmol CO₂ fixed m⁻² s⁻¹). Further, the higher photosynthetic rates came at the expense of generally higher dark respiration rates and generally higher light compensation points averaged over all environments. It is predicted that based on these light response curves and light levels along the Middle Rio Grande river riparian understory that Russian knapweed will be problematic under restored cottonwood habitat because of its tolerance for low light levels while perennial pepperweed may expand wherever over-story canopy is absent or sparse.

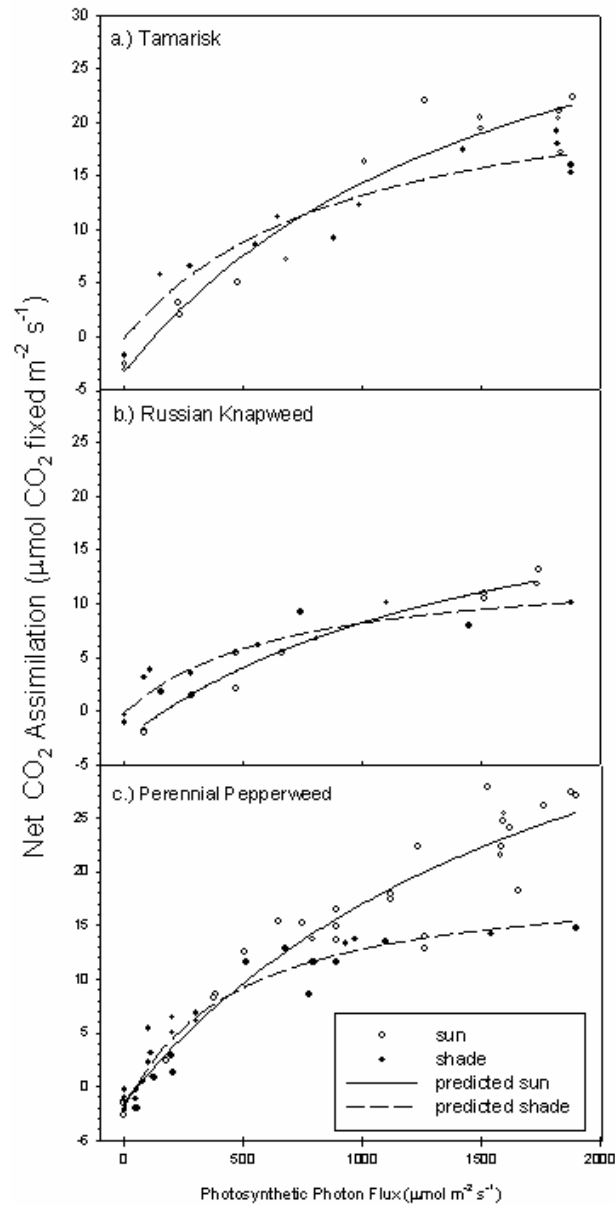


FIGURE 4. Net carbon dioxide assimilation ($\mu\text{mol CO}_2 \text{ fixed m}^{-2} \text{ s}^{-1}$) as a function of photosynthetic photon flux ($\mu\text{mol m}^{-2} \text{ s}^{-1}$) for tamarisk (a), Russian knapweed (b), and perennial pepperweed (c) grown without overstory shade (sun, o) and grown under shade (shade, ●). Rectangular hyperbolic functions were fit to the observed data with those parameters presented in Table 1.

TABLE 3. Photosynthetic parameter estimates (\pm SE) from equation 2 for tamarisk, Russian knapweed, and perennial pepperweed grown without over story shade (sun) and under over story shade (shade). The parameter R represents the estimated dark respiration rate, P_{\max} represents the estimated light saturated photosynthetic rate, K is the light level at which photosynthesis is half saturated, and r^2 is the coefficient of determination.

	growth	R	P_{\max}	K	r^2
Tamarisk	sun	-3.21 ± 1.60	46.8 ± 17.0	1658 ± 897	0.94
	shade	-0.14 ± 1.76	25.9 ± 4.4	937 ± 489	0.91
Russian knapweed	sun	-2.66 ± 1.09	28.6 ± 7.0	1623 ± 930	0.96
	shade	-0.15 ± 0.93	13.9 ± 2.9	665 ± 392	0.86
Perennial pepperweed	sun	-1.47 ± 1.46	55.3 ± 12.6	1989 ± 878	0.91
	shade	-2.14 ± 0.66	21.7 ± 1.7	456 ± 116	0.93

GROWTH ANALYSIS

Perennial pepperweed plants that were established in the greenhouse at Cal Poly as described in the photosynthesis section were used for a growth analysis. Above ground plant portions were sampled 14, 23, 33, 44, 63 and 96 days after establishment and were separated according to leaves and stems. Petioles were designated as stem material because they intercept light in a similar manner to stems. Leaf area was measured using a Cannon L-30 Scanner, images were stored as bit map images and then analyzed with Scion software to assess leaf area (cm^2). Then all samples plant parts were oven dried at 80°C . The classical interval approach to growth analysis was chosen (Chiariello et al. 1991). Biomass production per unit of current biomass, or RGR, can be defined in terms of the net assimilation rate (NAR) and leaf area ratio (LAR):

$$RGR = NAR * LAR$$

$$\frac{dw(t)}{dt} \left(\frac{1}{w(t)} \right) = \frac{dw(t)}{dt} \left(\frac{1}{a(t)} \right) * \left(\frac{a(t)}{w(t)} \right) \quad \text{eq. 3}$$

where $w(t)$ and $a(t)$ are shoot biomass and leaf area at time, t , respectively. NAR is indicative of the efficiency of the assimilatory organs to produce biomass, and LAR may be viewed as an indicator of plant 'leafiness'. LAR can be further decomposed into specific leaf area (SLA), a measure of leaf thickness, and leaf weight ratio (LWR), a measure of the leaf weight fraction of the shoot:

$$LAR = SLA * LWR$$

$$\frac{a(t)}{w(t)} = \left(\frac{a(t)}{l(t)} \right) * \left(\frac{l(t)}{w(t)} \right) \quad \text{eq. 4}$$

where $l(t)$ is leaf weight at time, t , and all other terms are defined above.

The growth analysis revealed patterns of growth among perennial pepperweed plants grown at different shade levels that are consistent with plants that can acclimate well to shaded conditions (Figures 5 and 6). Plants in unshaded (sun) conditions maintained higher, though not significantly different, relative growth rates (RGR) throughout the season than plants under either of the shade conditions (Figure 5a). Decomposing RGR reveals that the higher RGR of unshaded (sun) plants may have been due to higher net assimilation rates (NAR) throughout the season that were significantly higher on several dates than the NARs of plants in the shaded conditions. Further decomposition of leaf area ratio (LAR) reveals that leaves adjusted to

significantly to their shaded condition by possessing a significantly higher specific leaf area (SLA) or thinner leaves (Figure 6a). Producing thinner leaves is a typical response of plants grown in shaded conditions as described in Steinmaus and Norris (2002) and may be a characteristic that distinguishes invasive species from noninvasive species (Grotkopp et al., 2002).

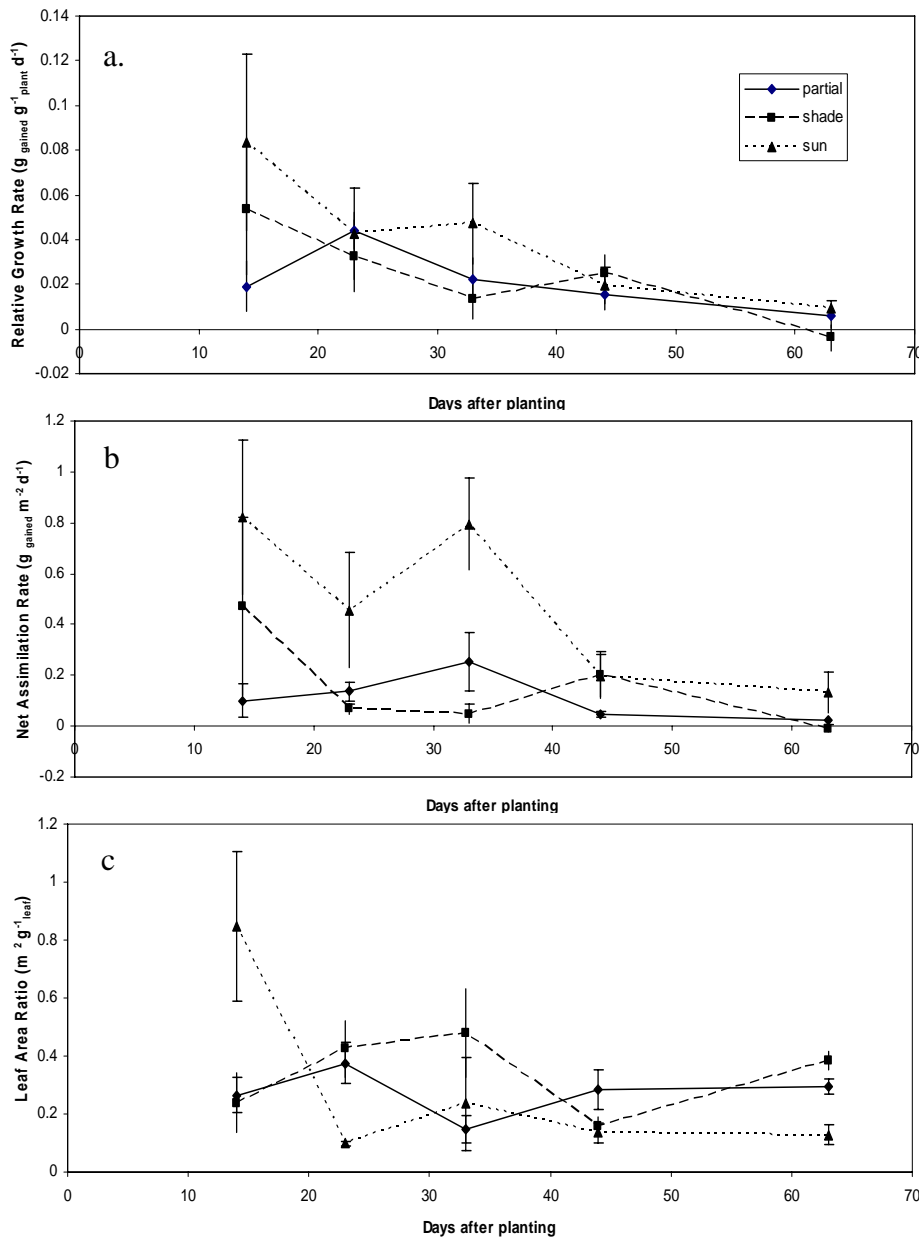


FIGURE 5. Growth analysis for perennial pepperweed. Relative growth rate (a) decomposed into net assimilation efficiency (b) and leaf area ratio (c) grown in full sun, partial shade (under 50% shade) and under shade (80% shade). Bars represent the standard error of the mean at each date.

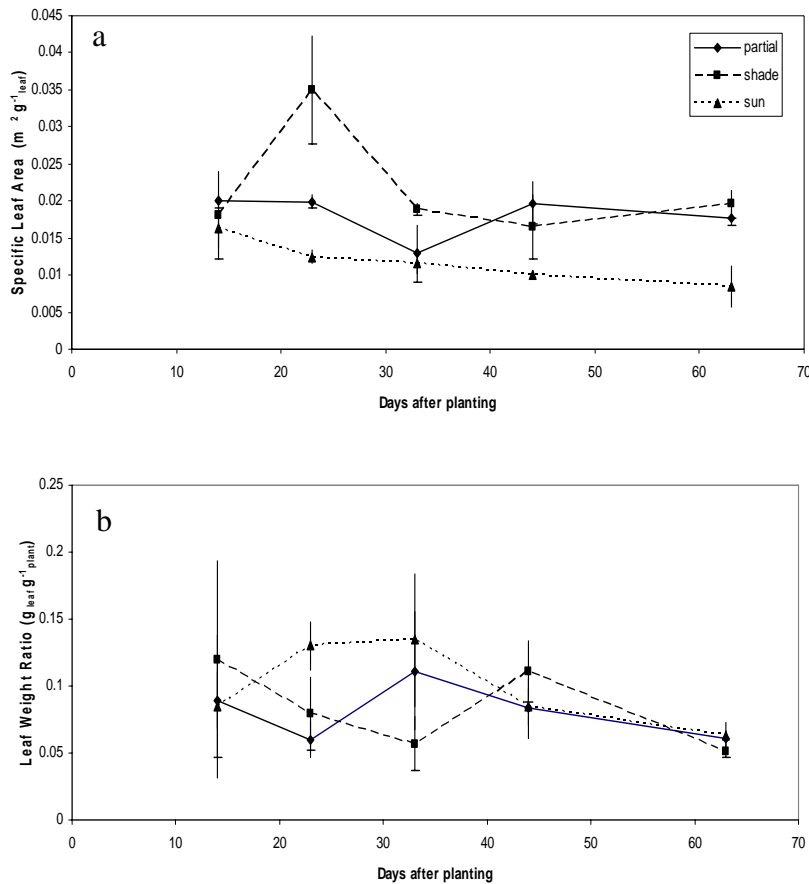


FIGURE 6. Decomposition of leaf area ratio into specific leaf area ($\text{m}^2 \text{g}^{-1} \text{leaf}$) and leaf weight ratio ($\text{g leaf g}^{-1} \text{plant}$) into specific leaf area (a) and leaf weight ratio (b) for perennial pepperweed plants grown in full sun, partial shade (under 50% shade) and under shade (80% shade). Bars represent the standard error of the mean at each date.

OBJECTIVE #4: Evaluate the efficacy of herbicide treatments to perennial pepperweed & Russian knapweed when grown under various light levels

The Russian knapweed crowns never established at Cal Poly in San Luis Obispo, so we conducted herbicide efficacy trials only on perennial pepperweed. Over 100 perennial pepperweed plants remained following the photosynthesis and growth analysis portion of this project. As discussed in objective 3, they had been established from crown-root fragments in 1 and 5 gallon pots using sterilized potting media at California Polytechnic State University in San Luis Obispo during the summer and fall of 2006. These pots were randomly divided into three shade conditions representative of the light conditions that might be experienced by under-story plants along the Middle Rio Grande River riparian habitat: unshaded, partially shaded (50% shade), and shaded (80% shade). Herbicide treatments consisted of 2% a.e. glyphosate (Roundup/Rodeo), 1% a.i. imazapyr (Arsenal/Habitat), and an untreated control. Plants were assessed for vigor at 18 days after application. Pairwise comparisons at the 5% and 1% significance levels were made of each herbicide/light condition with the controls. Plants from all

light conditions treated with either of the herbicides suffered some vigor reduction most often exhibited as chlorosis leading to necrosis in extreme cases and some growth deformation (Figure 7). Plants from all light levels that were treated with 1% glyphosate suffered reduced vigor relative to the untreated control plants grown in those same light conditions at least at the 5% significance level (Table 4). Plants grown in the fully shaded condition (80% shade) suffered highly significant reductions at the 1% significance level. Only perennial pepperweed plants grown in full shade conditions and treated with imazapyr suffered significant vigor reductions at the 5% significance level.

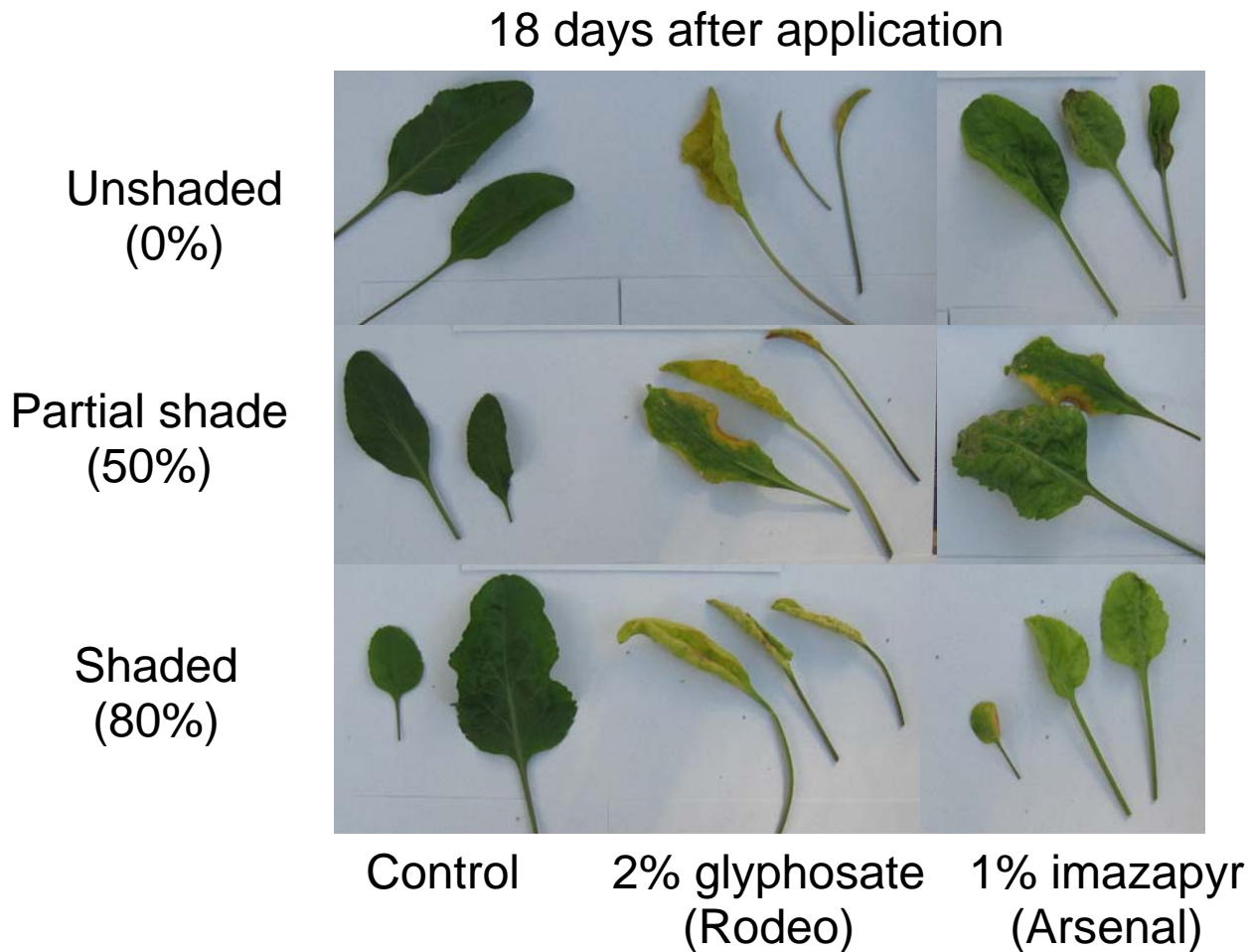


FIGURE 7. Perennial pepperweed leaves 18 days after treatment with 2% glyphosate (Rodeo), 1% imazapyr (Arsenal), and water (control) grown in unshaded, partial shade (50%), and shaded (80%) conditions.

TABLE 4. Vigor ratings for perennial pepperweed plants after 18 days of being treated with 1% glyphosate (Rodeo) and 2% imazapyr (Arsenal) grown in unshaded, partially shaded (50%), and shaded (80%) conditions relative to untreated control leaves grown under the same shade conditions. A single asterisks is indicative of a significant difference between treated and untreated (control) leaves at the 5% level and two asterisks are indicative of a difference at the 1% level.

	<u>Vigor (10=equal to control, 0=death)</u>	
	1% glyphosate	2% imazapyr
unshaded	4.3 *	7.5
partial	6.0 *	6.8
shade	3.8 **	6.2 *

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2.) FUTURE OF PROJECT:

Dr. Renz has accepted a position at the University of Wisconsin, leaving New Mexico State University. Dr. Steinmaus is in California so both investigators are now some distance away from New Mexico. However, this project has stimulated significant interest in conducting future research projects in communities where invasive phreatophytic species such as tamarisk are being removed and the potential for under-story invasives to move in is high. These situations are common in California where Steinmaus currently resides, and often involve the same invasive species (tamarisk and perennial pepperweed). The approach and results are very promising in terms of providing recommendations to restoration ecologists as to what they can anticipate, and what they need to achieve in terms of light suppression before they can walk away from a "restored" ecosystem. The data we generated is worthy of publication and steps are being taken to insure that it happens. This data should also be sufficient to justify future funding on a larger scale.

DISSEMINATION

Steinmaus, S. and M. Renz. in press. Perennial Pepperweed and Russian Knapweed. Invasion Following Tamarisk Removal Along the Rio Grande River. Proceedings to the Western Society of Weed Science (Presented at Annual Meetings, Portland OR March 14, 2007).

3.) STATEMENT OF CIPM FUNDING VALUE:

This funding provided the impetus for collaboration between two invasive plant ecologists, one whose research is more applied and the other more basic. This union brings together two very robust approaches to solve a very complex problem. This is the kind of multifaceted approach that we believe is required to meet the multifaceted challenges of riparian restoration west of the 100th meridian especially when it involves a phreatophyte such as tamarisk and a low growing invasive such as perennial pepperweed.

4.) BUDGET

Category	Total
Salary Undergraduate students to assist with planting, watering, and maintenance of experiments throughout summer	\$ 1,000
Supplies Pots, soil, fertilizer, shade cloth, wood to build shade frames, stakes for field plots	\$ 459
Travel Travel remote locations to view infestations under Tamarix canopies	\$ 500
Indirect (10%)	\$ 180
Subaward to Cal Poly. SLO	
Salary Faculty compensation for Compiling data collected at field sites in August 2005 Data analysis for further experimental designs Establishment of greenhouse trials Data Collection for growth analysis Data collection for photosynthesis and fluorescence Data analysis for growth analysis and photosynthesis	\$ 2,831
Total	\$ 4,970