A. Grant Data

- Title: **Integrated Control of Spotted Knapweed: Utilizing Spotted Knapweed-Resistant Native Plants to Facilitate Revegetation**
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  - Tiffany L. Weir, PhD student, Colorado State University.
  - Mathew Schultz, MS student, Colorado State University.
- States involved: Colorado, Montana
- First year funded and number of years funded: 2005, 3 years
- Total funding amount: $160,000

B. Nontechnical Summary.

Affordable long-term methods of weed control are lacking for many of North America's most destructive exotic invasive plants, including the knapweeds (spotted, diffuse, Russian). Recent research suggests that some exotic weeds are so successful because they produce potent toxins that are novel to the native species in their invaded range. In particular, the use of novel chemical weapons has been implicated as a mechanism of invasion for spotted, diffuse, and Russian knapweed. We argue that integrated research strategies that recognize the importance of these chemicals in invasions can suggest completely new approaches to fighting exotic invaders.

Specifically, we propose that native North American species that are resistant to knapweed chemicals may be used to detoxify knapweed chemicals and facilitate establishment of diverse native communities in knapweed-infested sites. Through previous work funded by WR-IPM grant no. 2003-05060, we identified North American grassland species that are resistant to the toxin produced by spotted knapweed. Further, we discovered that some of these resistant species detoxify chemicals by secreting organic acids into the soil. These organic acids in turn appear to protect neighboring susceptible plants from the toxic effects of knapweed toxins. Based on these results, we proposed to test whether planting native species that are resistant and secrete large quantities of organic acids can be used as a method to facilitate replacement of spotted knapweed monocultures with diverse native communities. If so, our research would suggest that constitutive and evolved resistance in native species to the chemical effects of invaders could be used as a low-cost, selective approach to management of sites infested with some weeds.

We conducted greenhouse and field experiments to examine whether planting native species that accumulate organic acids in the soil increases the success of susceptible native species in the presence of spotted knapweed. In addition, we examined other resistant species for organic acid secretion or other mechanisms of chemical resistance, to identify further species that might be used to facilitate native species establishment in spotted knapweed fields.

C. Introduction.

Affordable long-term methods of weed control are lacking for many of North America's most destructive exotic invasive plants, including the knapweeds (*Centaurea maculosa, C. diffusa, Acroptilon repens*). Recent research suggests that some exotic weeds are so successful because they produce potent phytotoxins (i.e., allelochemicals) that are novel to the native species in their invaded range. In particular, allelopathy has been implicated as a mechanism of invasion for spotted, diffuse, and Russian knapweed. We proposed that integrated research strategies that recognize the importance of allelochemicals in invasions can suggest completely new approaches to fighting exotic invaders.

Specifically, we explored whether native North American species that are resistant to knapweed allelopathy could be used to detoxify knapweed allelochemicals and facilitate establishment of diverse native communities in knapweed-infested sites. Through previous work funded by WR-IPM grant no. 2003-05060, we identified North American grassland species that are resistant to the phytotoxin produced by spotted knapweed, (±)-catechin. Further, we discovered that some of these (±)-catechin-resistant species detoxify (±)-catechin by secreting organic acids into the soil. These organic acids in turn appear to protect neighboring (±)-catechin-susceptible plants from the toxic effects of (±)-catechin. Based on these results, we proposed to test whether planting native species that are (±)-catechin-resistant and rhizosecrete large quantities of organic acids can be used as a method to facilitate replacement of spotted knapweed monocultures with diverse native communities. If so, our research would suggest that constitutive and evolved resistance in native species to the allelopathic effects of invaders could be used as a low-cost, selective approach to management of sites infested with allelopathic weeds.

We conducted greenhouse and field experiments to examine whether planting native species that accumulate organic acids in the soil increases the success of (±)-caetchin-susceptible native species in the presence of spotted knapweed. In addition, we examined other species identified as (±)-catechin resistant for organic acid secretion or other mechanisms of (±)-catechin resistance, to identify additional species that might be used to facilitate native species establishment in spotted knapweed fields. Using allelochemical-resistant plants to facilitate establishment of native communities in weed-infested sites would reduce our reliance on synthetic treatments, and add to the arsenal of insect biocontrol, cultural practices, and mechanical methods we currently have available for integrated weed management systems.

D. Objectives.

1) **To determine if plants that excrete high concentrations of organic acids into the rhizosphere (i.e., silky lupine and common blanketflower) can be used to detoxify spotted knapweed soils and allow for the subsequent establishment of native vegetation. The native vegetation examined included several catechin-resistant and catechin-sensitive species that we have screened and characterized in previous studies.**

A putative catechin-sensitive native species, Idaho fescue, was grown in a greenhouse study with or without spotted knapweed or exogenous catechin, and with or without catechin-resistant plants or exogenous oxalic acid. Catechin-resistant species and oxalic acid treatment did not improve fescue biomass, suggesting that these resistant species are not good nurse crops for encouraging native grassland species in competition with knapweed. However, we have also conducted field experiments where we applied different native plant seed mixtures to areas infested with Russian knapweed and spotted knapweed. In these field studies, our results indicate that seeding native species that are good competitors, or potentially resistant to allelopathic effects, increase the diversity and abundance of native plants. However, it is yet to be seen if this increase in natives will reduce exotic invasion. Field studies from this project will continue to be monitored for several years using other funding sources.

2) **To identify which of the (±)-catechin-resistant plants identified in the previous funding cycle also produce high concentrations of (±)-catechin-fighting organic acids, like blanketflower and silky lupine.**

The results from these lab experiments show that there was no correlation between oxalic acid secretion and catechin resistance. Most of the seedlings tested secreted little or no detectable oxalic acid and there was more often oxalic acid present in the root exudates of plants not exposed to catechin than to those that had been exposed to the phytotoxin. It is still possible that the secretion of other antioxidants, such as certain flavonoid compounds may be determining factors in the increased resistance to catechin that is seen in some native plants. However, the secretion of oxalic acid, as seen in *Gaillardia* and *Lupinus sp*. does not appear to be a general mechanism of catechin resistance.

E. Approach.

A combination of greenhouse and field experiments were used to address Objective 1. The greenhouse experiment was used to examine (under controlled conditions) the consequences of organic acid accumulation in the soil for the success of native species in the presence of spotted knapweed and its allelochemical, catechin. The field experiments are exploring the effects of native species that produce organic acids on the success of diverse native communities under realistic conditions for revegetation of spotted knapweed infested areas.

For Objective 2, we used laboratory assays to evaluate constitutive and elicited organic acid production in several catechin-resistant North American species that were previously identified. We conducted *in vitro* experiments with these species to examine whether the organic acids or other compounds present in their root exudates can protect catechin-sensitive species from the effects of catechin treatment as previously described. We grew catechin-sensitive species, in liquid growth media with either catechin or catechin plus root exudates of catechin-resistant species in order to see if catechin-resistant species are able to reduce catechin toxicity.

F. Results.

Objective 1. Lab and greenhouse experiments determined that catechin-resistant species, and oxalic acid treatment did not improve the biomass of a senstivie species (Idaho fescue) when in competition with knapweed or when treated with catechin, suggesting that catechin-resistant species are not affective nurse crops for encouraging native grassland species in competition with knapweed. Several lines of evidence suggest that catechin did not influence knapweed-fescue interactions in our experiment. Activated carbon did not improve fescue growth in the presence of knapweed, suggesting that organic compounds from knapweed did not inhibit fescue. Catechin applied exogenously appeared to degrade rapidly and had no phytotoxic effect, and in situ catechin production was episodic, all of which may have limited opportunities to observe allelopathy and facilitation.

We have futher explored Objective 1 in several field experiments and have set-up a number of other field and lab experiments that will continue producing data for multiple seasons. These experiments go well beyond our proposed research. We have completed experiments where we applied different native plant seed mixtures to areas infested with Russian knapweed and spotted knapweed. We are currently analyzing 2008 data, but generally our results indicate that seeding native species that are good competitors, or potentially resistant to allelopathic effects, increase the diversity and abundance of native plants. However, it is yet to be seen if this increase in natives will reduce exotic invasion. This is a question that can only be answered over a longer time-frame. We will continue to prepare these results to submit for peer review publications as the data becomes available.

Objective 2. We hypothesized that secretion of oxalic acid or other organic acids may have been partially responsible for resistance against the oxidative damage caused by catechin secreted from spotted knapweed. However, the results from our experiments conducted for Objective 2 show that there was no correlation between oxalic acid secretion and catechin resistance. Most of the seedlings tested secreted little or no detectable oxalic acid and there was more often oxalic acid present in the root exudates of plants not exposed to catechin than to those that had been exposed to the phytotoxin. It is still possible that the secretion of other antioxidants, such as certain flavonoid compounds may be determining factors in the increased resistance to catechin that is seen in some native plants. However, the secretion of oxalic acid, as seen in some catechin-resistant species does not appear to be a general mechanism of catechin resistance. Given these results, we have turned our attention to determining whether or not different competitors would provoke different responses at the level of gene expression in spotted knapweed, in order to better understand the mechanistic basis of plant-plant competiton. Spotted knapweed was grown by itself or in competition with catechin-sensitive Idaho fescue, or with catechin-resistant blanket flower. We hybridized knapweed cDNAs from each of these competitive situations to an *Arabidopsis* microarray chip, and followed this analysis with quantitative PCR on a subset of genes. We found that some transcripts were up or down regulated regardless of the plant neighbor grown with knapweed, whereas other transcripts showed differential expression related to the competitor species identity. These changes indicate both general and species-specific aspects of knapweed's competitive response to different plant neighbors at the level of gene expression. By further characterizing this system we hope to better understand the mechanistic basis of species specific competition, and identify molecular factors that may facilitate plant invasion.

G. Impacts.

Previous work by our research team and others has indicated that invasive knapweeds have evolved a strategy in which allelochemicals play a critical role in their invasion success. In this study, we hypothesized that some plants possess effective counter-chemical strategies to resist knapweed, which could be exploited in order to control knapweed invasions. Our work focused on exploring the use of knapweed-resistant native species for the integrated management of spotted knapweed. Along the way, we have further examined the mechanisms underlying the interactions between knapweeds and native species that appear to be successful competitors. Our greenhouse and lab studies have indicated that the nature of below-ground chemical interactions between knapweeds and native plants are complex, conditional, and episodic. Nevertheless, several of our field studies have indicated that planting native species, which have been shown to be resistant to knapweed phytotoxins, can increase the diversity and abundance of native plants within knapweed infestations. We are hopeful that this knowledge could eventually lead to the development of better revegetation regimes utilizing native plants that will tolerate knapweed chemical interference and eventually facilitate the establishment of desirable vegetation. Such knowledge would lessen dependence on herbicides for the control of knapweeds.

Results from these studies have the potential to lead to an innovative and novel approach for the management of some of the most problematic and extensive weed infestations in Western

North America. As the field studies established under this project mature, we will make recommendations regarding seed mixtures and species that would be useful for revegetating infestations of spotted and Russian knapweeds. This approach might be extended to other known, or yet to be discovered, allelopathic invasive species. Results from these studies could be rapidly assimilated and utilized by land managers because our approach involves existing methodologies (seeding) and does not necessitate additional equipment or expenditures. Rather, implementing results from these studies involves a switch to more effective revegetation species. Such weed-combating seed mixtures would reduce control costs because competitive plant communities should hinder re-invation by weeds and would thus lessen the need for additional management actions while at the same time, restore value to degraded rangelands.

Results from this research have been transmitted to the scientific and land management community through, publication in professional peer-reviewed scientific journals and presentations at professional symposia. A list of publications and presentations follows:

<u>Publications</u>

Schultz, M.J., L.G. Perry, J.M. Vivanco and M.W. Paschke. Effects of (±)-catechin-resistant species on (±)-catechin-sensitive species in interactions with Centaurea stoebe. In preparation for submission to Journal of Applied Ecology.

Broz, A.K. R.M. Callaway, M.W. Paschke, D.K. Manter and J.M. Vivanco. 2008. A molecular approach to understanding plant-plant interactions in the context of invasion biology. Functional Plant Biology (*In press*).

Broeckling, C.D., Manter, D.K., Paschke, M.W., and Vivanco, J.M. (2008) Rhizosphere ecology. In Sven Erik Jorgensen and Brian D. Fath (Editor-in-Chief), General Ecology. Vol. [4] of Encyclopedia of Ecology, 5 vols. pp. [3030-3035] Oxford: Elsevier.

Alford, É.R., J.M. Vivanco and M.W. Paschke. 2008. The effects of flavonoid allelochemicals from knapweeds on legume - rhizobia candidates for restoration. Restoration Ecology (*DOI: 10.1111/j.1526-100X.2008.00405.x*).

Schultz, M.J. 2008. Soil Ecological interactions of spotted knapweed and native plant species. M.S. Thesis. Colorado State University, Graduate Degree Program in Ecology. 66pp.

Perry, L.G, Alford, E.R., Horiuchi, J., Paschke, M.W., and Vivanco, J.M. (2007). Chemical signals in the rhizosphere: root-root and root-microbe communication. In The Rhizosphere (Second Edition). Pinton, R., Varanini, Z. and P. Nannipieri, eds. CRC Press. Boca Raton, FL. pp. 297-330.

Prithiviraj, B., Paschke, M.W., and Vivanco, J.M. (2007). Root communication: the role of root exudates. Encyclopedia of Plant and Crop Science 1:1, 1-4 DOI: 10.1081/E-EPCS-120042072

Broz, A. K., Vivanco, J. M., Schultz, M. J., Perry, L. G., and Paschke, M. W. (2006) Secondary Metabolites and Allelopathy in Plant Invasions: A Case Study of Centaurea maculosa. In Plant Physiology, Fourth Edition, L. Taiz and E. Zeiger, eds. On line Essay 13.7. http://4e.plantphys.net/article.php?ch=e&id=377

Inderjit, Callaway, R.M., and Vivanco, J.M. (2006) Can plant biochemistry contribute to understanding of invasion ecology? Trends in Plant Sciences 11:574-580

Weir, T.L., Bais, H.P., Stull, V.J., Callaway, R.M., Thelen, G.C., Ridenour, W.M., Bhamidi, S., Stermitz, F.R., and Vivanco, J.M. 2006. Oxalate contributes to the resistance of Gaillardia grandiflora and Lupinus sericeus to a phytotoxin produced by *Centaurea maculosa*. Planta 223:785-795.

Perry, L.G., Johnson, C., Alford, E.R., Vivanco, J.M., and Paschke, M.W. 2005. Screening of grassland plants for restoration after spotted knapweed invasion. Restoration Ecology 13:725-735

Presentations

Paschke, M.W. 2008. Revegetation workshop: Restoration and soil ecology. Workshop organizer / teacher. Eighteenth high altitude revegetation workshop, March 2008, Rocky Mountain National Park.

Paschke, M.W. 2007. Using novel weapons to restore weed-infested plant communities. **Invited seminar**, Colorado State University, Department of Forest Rangeland and Watershed Stewardship. April 11, 2007

Paschke, M.W. 2007. Managing soil processes for ecological restoration and exotic weed control. **Invited talk** at the 2007 Colorado AES Research Center Conference. January 11, 2007, Fort Collins, CO.

Rieder, J.P., M.W. Paschke, L.G. Perry, S.A. Cronin, R.M. Callaway and J.M. Vivanco. 2007. Allelopathy and the control of exotic weeds on military training grounds. The 2007 SERDP and ESTCP Partners in Environmental Technology Technical Symposium, December 2007, Washington, D.C.

Schultz, M.J., L.G. Perry and M.W. Paschke. 2007. Importance of establishment order in competitive interactions between native plant species and *Centaurea stoebe.* ESA/SER Joint meeting, August 2007, San Jose, CA.

Cronin, S.A., M.W Paschke, L.G Perry, E.F Redente, and J.M Vivanco. 2007. Using native allelopathic species to combat exotic species. ESA/SER Joint meeting, August 2007, San Jose, CA.

Paschke, M.W. 2006. Restoration from the ground up: Understanding and managing below-ground processes for invasive weed control. **Invited talk** at the 2006 SERDP and ESTCP Partners in Environmental Technology Technical Symposium. November 2006, Washington, D.C.

Paschke, M.W. Using novel weapons to control invasive plants. **Invited seminar** at the University of Denver, Department of Biological Sciences. November 2006. Denver, Colorado.

Paschke, M.W. Novel weapons and the battle against alien plant species. **Invited seminar** at the University of Alicante. September, 2006. Alicante, Spain

Paschke, M.W. Restoration from the ground up: Manipulating plant community succession via soil processes. **Invited seminar** at the Fundación Centro de Estudios Ambientales del Mediterráneo. September, 2006. Valencia, Spain

Paschke, M.W., L.G. Perry, S.A. Cronin, J.P. Rieder, J.M. Vivanco, R.M. Callaway, K. Szitár and K. Török. Using novel weapons to control exotic species. **Invited talk** at the 1st European Congress of Conservation Biology. August 2006 – Eger, Hungary.

Callaway, R.W., T.Weir, L.Perry, G.C. Thelan, M. Paschke and J. Vivanco. 2006. Developing weed resistant plant communities. The 2006 SERDP and ESTCP Partners in Environmental Technology Technical Symposium, November 2006, Washington, D.C.

Rieder, J.P., M.W. Paschke, L.G. Perry, S.A. Cronin, R.M. Callaway, J.M. Vivanco. 2006. Novel weapons for controlling exotic plant species. The 2006 SERDP and ESTCP Partners in Environmental Technology Technical Symposium, November 2006, Washington, D.C.

Alford, É.R, L.G. Perry, M.W. Paschke and J.M. Vivanco. Tough enough? Identification of allelochemical resistant plants for revegetation following spotted knapweed invasion. The 2005 SERDP and ESTCP Partners in Environmental Technology Technical Symposium, December 2005, Washington, D.C.

Perry, L.G. C. Broeckling, M.W. Paschke and J.M. Vivanco. Variation in production of the phytotoxin (±)-catechin by the invasive spotted knapweed, effects on knapweed and implications for management. The 2005 SERDP and ESTCP Partners in Environmental Technology Technical Symposium, December 2005, Washington, D.C.
Vivanco, J.M., B. Qin, L.G. Perry, M.W. Paschke and F. Stermitz. Identification of phytotoxins in the root exudates of exotic invasive weeds on military training lands. The 2005 SERDP and ESTCP Partners in Environmental Technology Technical Symposium, December 2005, Washington, D.C.
Callaway, R.M., J.M. Vivanco and M.W. Paschke. Natural selection for resistance in natives to the allelopathic effects of invasive plants. The 2005 SERDP and ESTCP Partners in Environmental Technology Technical Symposium, December 2005, Washington, D.C.