References

Allison, V.J., R.M. Miller, J.D. Jastrow, R. Matamala and D.R Zak. 2005. Changes in soil microbial community structure in a tallgrass prairie chronosequence. *Soil Science Society of America Journal* 69:1412–1421.

Anderson M.J. 2001. A new method for non parametric multivariate analysis of variance. *Austral Ecology* 26:32–46.

Bach E.M., S.G. Baer, C.K. Meyer and J. Six. 2010. Soil texture affects soil microbial and structural recovery during grassland restoration. *Soil Biology and Biochemistry* 42:2182–2191.

Boerner, R.E.J., B.G. DeMars and P.N. Leicht. 1996. Spatial Patterns of mycorrhizal infectiveness of soils long a successional chronosequence. *Mycorrhiza* 6:79–90.

Bray, R.H. and L.T. Kurtz. 1945. Determination of total organic and available forms of phosphorus in soils. *Soil Science* 59:39–45.

Bray, J.R. and J.T. Curtis. 1957. An ordination of the upland forest community of Southern Wisconsin. *Ecology Monographs* 27: 325–349.

Busby, R.R., D.L. Gebhart, M.E. Stromberger, P.J. Meiman and M.W. Paschke. 2011. Early seral plant species' interactions with an arbuscular mycorrhizal fungi community are highly variable. *Applied Soil Ecology* 48:257–262.

Caporaso, J.G., J. Kuczynski, J. Stombaugh, K. Bittinger, F.D. Bushman, E.K. Costello et al. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7: 335–336.

de Mendiburu, F.2016. Agricolae statistical procedures for agricultural research. R package version 1.2–4. CRAN.R-project.org/package=agricolae.

Dhillion, S.S. and C.F. Friese. 1992. The occurrence of mycorrhizas in prairies: Application to ecological restoration. *Proceedings of the Thirteenth North American Prairie Conference: Spirit of the Land Our Prairie Legacy.* Windsor Ontario: Department of Parks and Recreation.

Edgar, R.C. 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26:2460–2461.

Hart, M.M., K. Aleklett, P.-L. Chagnon, C. Egan, S. Ghignone, T. et al. 2015. Navigating the labyrinth: A guide to sequence-based community ecology of arbuscular mycorrhizal fungi methods. *New Phytologist* 207:235–247.

Hartnett D.C. and G.W.T. Wilson. 1999. Mycorrhizae influence plant community structure and diversity in tallgrass prairie. *Ecology* 80:1187–1195.

Helgason, T., T.J. Daniell, R. Husband, A.H. Fitter and J.P. Young. 1998. Ploughing up the wood-wide web? *Nature* 394:431.

Helgason, T., A.H. Fitter and J.P.W. Young. 1999. Molecular diversity of arbuscular mycorrhizal fungi colonising *Hyacinthoides non-scripta* (bluebell) in a seminatural woodland. *Molecular Ecology* 8:659–666.

Hijri, I., Z. Sýkorová, F. Oehl, K. Ineichen, P. Mäder, A. Wiemken and D. Redecker. 2006. Communities of arbuscular mycorrhizal fungi in arable soils are not necessarily low in diversity. *Molecular Ecology* 15:2277–2289.

Jansa, J., A. Wiemken and E. Frossard. 2006. The effects of agricultural practices on arbuscular mycorrhizal fungi. *Geological Society London Special Publications* 266:89–115.

Johnson, N.C., D.R. Zak, D. Tilman and F.L. Pfleger. 1991. Dynamics of vesicular-arbuscular mycorrhizae during old field succession. *Oecologia* 86:349–358.

Johnson, N.C., G.W.T. Wilson, M.A. Bowker, J.A. Wilson and R.M. Miller. 2010. Resource limitation is a driver of local adaptation in mycorrhizal symbioses. *Proceedings of the National Academy of Sciences USA* 107:2093–2098.

Lee, J., S. Lee and J.P.W. Young. 2008. Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. *FEMS Microbiology Ecology* 65:339–349.

Lekberg, Y., T. Schnoor, R. Kjøller, S.M. Gibbons, L.H. Hansen, W.A. Al-Soud, et al. 2012. 454–Sequencing reveals stochastic local reassembly and high disturbance tolerance within arbuscular mycorrhizal fungal communities. *Journal of Ecology* 100:151–160.

Ohsowski, B.M., J.N. Klironomos, K. Dunfield and M.M. Hart. 2012. The potential of soil amendments for restoring severely disturbed grasslands. *Journal of Applied Soil Ecology* 60:77–83.

Oehl, F., E. Sieverding, P. Mäder, D. Dubois, K. Ineichen, T. Boller and A. Wiemken. 2004. Impact of long-term conventional and organic farming on the diversity of arbuscular mycorrhizal fungi. *Oecologia* 138:574–583.

Opik, M., A. Vanatoa, E. Vanatoa, M. Moora, J. Davison, J.M. Kalwij, et al. 2010. The online database MaarjAM reveals global and ecosystem distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). *The New Phytologist* 188:223–241.

Paulson, J.N., O.C. Stine, H.C. Bravo and M. Pop. 2013. Differential abundance analysis for microbial marker-gene surveys. *Nature Methods* 10:1200–1202.

Richter, B.S. and J.C. Stutz. 2002. Mycorrhizal inoculation of big sacaton: implications for grassland restoration of abandoned agricultural fields. *Restoration Ecology* 10:607–616.

Rodger, L. 1998. Tallgrass communities of Southern Ontario: a recovery plan. World Wildlife Fund and the Ontario Ministry of Natural Resources.

Smith, S. and D. Read. 2008. *Mycorrhizal Symbiosis 3rd Edition*. London, UK: Academic Press.

Stover, H.J., R.G. Thorn, J.M. Bowles, M.A. Bernards and C.R. Jacobs. 2012. Arbuscular mycorrhizal fungi and vascular plant species abundance and community structure in tallgrass prairies with varying agricultural disturbance histories. *Applied Soil Ecology* 60:61–70.

Zhang, T., Y. Sun, Z. Shi and G. Feng G. 2012. Arbuscular mycorrhizal fungi can accelerate the restoration of degraded spring grassland in Central Asia. *Rangelant Ecology and Management* 65:426–432.

Evaluation of Clopyralid and Additives for *Coronilla varia* Suppression in a Remnant Prairie (Wisconsin)

Craig A. Annen (Corresponding author: Integrated Restorations, LLC, Belleville, WI, annen00@aol.com), Jared A. Bland (Integrated Restorations, LLC, Belleville, WI), Amanda J. Budyak (Integrated Restorations, LLC, Belleville, WI) and Christopher D. Knief (Integrated Restorations, LLC, Belleville, WI).

Coronilla varia (crown vetch) is a perennial legume that has been extensively planted for erosion control along roadsides since the 1950s, and has become a widespread invasive species in grasslands throughout the Midwestern United States. *Coronilla varia* spreads through

Table 1. Summary of clonal propagation features present in southwestern Wisconsin populations of *Coronilla varia*.

Trait	Mean	SE
Number of stems	8.0	0.6
Number of crown buds	3.3	0.3
Number of rhizomes	2.0	0.6
Number of buds/rhizome	5.3	1.5
Number of lateral roots/plant	8.0	1.2
Number of stems arising from root-splitting	2.7	0.3

prolific seed production and several modes of vegetative expansion, including rhizomes, crown buds, and root-splitting (Klimešová and de Bello 2009, Losure et al. 2009). Once established, *C. varia* outcompetes and displaces native vegetation through shading and smothering (Tu 2003). *Coronilla varia* invasions have been directly linked to declines in at-risk plant species populations (Walck et al. 1999), decreased reproductive success in prairie plants (Molano-Flores 2014), and indirect reductions in insect pollinator diversity through loss of plant diversity (Hopwood 2008). Suppression of *C. varia* is a management priority in regions where it is problematic, yet only a limited number of studies are available to direct practitioners' efforts. We conducted complementary anatomical and field studies to develop an effective and cost-feasible method for reversing *C. varia* invasions in grassland communities. We first quantified *C. varia's* capacity for clonal expansion and examined aspects of its cellular anatomy relevant to the use of herbicides for suppression. Then, using our anatomical findings to refine our approach, we tested the effects of clopyralid (Transline®, Dow AgroSciences, Inc.) herbicide on *C. varia* in a four-year field experiment.

We began by collecting a mature *C. varia* plant from three separate populations within a one-mile radius of the field site in Green County, Wisconsin. Plant samples were rinsed of attached soil and pressed between sheets of herbarium blotter paper for drying. Baseline data on *C. varia* clonal propagation motifs were obtained from the CLO-PLA3 database (Klimešová and de Bello 2009; clopla.butbn.cas.cz). We counted the numbers of stems, crown buds, rhizomes, rhizome buds (apical and lateral), lateral roots, and the number of stems arising from root-splitting on each plant. Our findings (Table 1) were congruent with those reported for central European *C. varia* populations in the CLO-PLA3 database. This invasive species' capacity for rapid clonal expansion and resprouting after suppression efforts highlighted the need for a multiple-year approach. Next, we examined *C. varia* cellular anatomy with a dissecting microscope (40´), which revealed the presence of silica bodies in both leaflet and stem sections. Silica bodies are crystalline deposits of hydrated silica absorbed by roots that serve as a structural component and deterrent to herbivory (Dayanandan et al. 1983). Silica bodies were abundant near the midrib and within guard cells of *C. varia* leaflets, and throughout stem sections. We postulated that the abundance of silica bodies in *C. varia* leaves could diminish herbicide action by rendering leaf surfaces physically slippery, resulting in herbicide run-off prior to uptake. We reasoned that we could enhance chemical control of *C. varia* by adding a sticking additive to our herbicide mixtures.

The field site was a 3.4-ha dry-mesic prairie remnant located in Green County, Wisconsin (42.85° N, 89.74° W). Prior to management, *C. varia* had invaded this site from adjacent roadside populations and was established in large patches. Sporadic clumps of relic prairie forbs and grasses were present, but limited in distribution to areas not yet invaded by *C. varia*. Two-thirds of the remnant was treated with clopyralid annually in mid-June 2014–2017, while the remaining third was not sprayed and served as a control. The sprayed and unsprayed sections were separated by a regularly-mowed hiking trail. We selected the mid-June timing window based on patterns of axillary bud growth and carbohydrate reserve levels reported by Brann and Jung (1973). We decided on a four-year suppression program based on our anatomical investigation and on evidence for apical dominance in rhizomes reported in *C. varia* (Carlson 1965). Rhizome apical dominance compels multiple-year suppression efforts to reverse perennial species invasions (see Annen 2010).

We chose clopyralid over other herbicide active ingredients recommended for *C. varia* (glyphosate, 2,4-D, and triclopyr [Tu 2003, Symstad et al. 2002]) because of its low volatility and selective activity for composites and legumes. We further chose clopyralid because it is less expensive than aminopyralid, a related herbicide (Duncan 2014). Clopyralid was applied as a spot treatment at 0.4% (0.5 ounces/gallon, or 4 mL/liter) with 3-gallon backpack sprayers. We added a methylated seed oil/nonionic surfactant blend (Dyne-Amic®, Helena Chemical Company, Inc., Collierville, TN.) to herbicide mixtures at 1% (1.3 ounces/gallon, or 10 mL/liter) as a spreading agent and to dissolve leaf cuticles that present a barrier to herbicide uptake. To promote enhanced suppression, we added a sticking agent (Induce®, Helena Chemical Company, Inc., Collierville, TN) to herbicide mixtures at 2% (2.5 ounces/gallon, or 20 mL/liter). This sticking agent caused applied herbicide to physically stick to *C. varia* rather than running off treated surfaces due to their enhanced silica content. Aboveground portions of *C. varia* plants were sprayed to a point of 90–100% coverage to achieve maximum herbicide exposure. Spray coverage was determined with a spray pattern indicator dye (Sensi-Pro®, Sensient Colors, LLC, St. Louis, MO).

We sampled vegetation on 14 July 2017 in ten 0.5 m^2 quadrats placed randomly along two 15-meter transects in both the treated and untreated sections. Transects were parallel and separated within each section by a ten-meter

Table 2. Summary of clopyralid effects on *Coronilla varia* in the field experiment. Values are mean (±SE).

Response	Clopyralid-treated	Untreated
C. varia cover	2.5 (0.3)	75.5 (7.0)
Native prairie plant cover	68.5 (8.4)	7.5 (2.4)
Species density	6.0 (0.7)	2.5 (0.5)

buffer. In each quadrat, we estimated species density (the number of species/quadrat) and cover of each species in increments of 5%, with no overlap (maximum cover = 100%). Coefficient of conservatism (c) values were assigned to native species from Bernthal (2003) and floristic quality was estimated as $(\Sigma c/S) * (\sqrt{S})$.

Following four treatments, we observed that the majority of *C. varia* plants sampled in the treated quadrats were immature seedlings, indicating that mature plants had been eradicated and resurgence was occurring mostly from the seed bank and not from perennial reserves of existing plants. Cumulative species richness (native and non-native species) was 27 in the treated section and 10 in the untreated section (Table 2). Floristic quality was also higher in the treated section; twenty-one species sampled in this section were native prairie plants (mean c = 4.6, FQA = 19.5), including Wisconsin Special Concern *Pediomelum esculentum* (prairie turnip), while only seven species in the untreated section were native (mean c = 3.7, FQA = 9.8). Our suppression approach was relatively inexpensive; one gallon of spray mix (herbicides and additives) cost $2.50 and annual labor costs were approximately $295/ha. We conclude that the suppression method reported here is an effective, cost-feasible means to reverse a *C. varia* invasion and promote competitive release of native prairie vegetation in remnant prairies, provided a multiple-year effort is employed.

Acknowledgements

We thank Daniel and Calico Schmidt for access to their property for conducting this study, and Charles Bauer and Charles Beckwith for permission to collect plant samples from Rare Earth Farm Preserve. Funding for this project was provided by Integrated Restorations, LLC.

References

Annen, C.A. 2010. Prospects for disrupting rhizome apical dominance prior to chemical control of *Phalaris arundinacea*. *Ecological Restoration* 28:291–299.

Bernthal, T.W. 2003. Development of a Floristic Quality Assessment Methodology for Wisconsin. Wisconsin Department of Natural Resources Final Report to USEPA–Region V.

Brann, D.E. and G.A. Jung. 1973. Influence of cutting management and environmental variation on the yield, bud activity, and autumn carbohydrate reserve levels of crown vetch. *Agronomy Journal* 66:767–773.

Carlson, G.E. 1965. Photoperiodic control of adventitious stem initiation on roots. *Crop Science* 5:248–250.

Dayanandan, P., P.B. Kaufman and C.I. Franklin. 1983. Detection of silica in plants. *American Journal of Botany* 70:1079–1084.

Duncan, C. 2014. Fall herbicide application timing improves crown vetch (*Securigera varia*) control. *TechLine Invasive Plant News* August 29. techlinenews.com/articles/2014/fall-herbicide-application-timing-improves-crown-vetch-control.

Hopwood, J.L. 2008. The contribution of roadside grassland restorations to native bee conservation. *Biological Conservation* 141:2632–2640.

Klimešová, J. and F. de Bello. 2009. CLO-PLA: The database of clonal and bud bank traits of central European flora. *Journal of Vegetation Science* 20:511–516.

Losure, D.A., K.A. Moloney and B.J. Wilsey. 2009. Modes of crown vetch invasion and persistence. *American Midland Naturalist* 161:232–242.

Molano-Flores, B. 2014. An invasive plant species decreases native plant reproductive success. *Natural Areas Journal* 34:465–469.

Symstad, A.J. L. Temmen and E. Anderson. 2002. Mowing does not increase effectiveness of herbicide on crown vetch in tallgrass prairie (Illinois). *Ecological Restoration* 20:287–288.

Tu, M. 2003. Element Stewardship Abstract for *Coronilla varia* L. The Nature Conservancy Wildland Invasive Species Team.

Walck, J.L., J.M. Baskin and C.C. Baskin. 1999. Effects of competition from introduced plants on establishment, survival, growth, and reproduction of the rare plant *Solidago shortii* (Asteraceae). *Biological Conservation* 88:213–219.

Does Removal of Invasive Garlic Mustard Affect Eastern Red-backed Salamanders?

Geoffrey R. Smith, Department of Biology, Denison University, Granville, OH 43023, smithg@denison.edu.

One challenge facing land managers is the invasion of non-native plants (Westman 1990, D'Antonio and Meyerson 2002). Decisions on remediation and removal of non-native plants need to be placed into a cost-benefit context, taking into account the costs and impacts of removal since not all invasive plants have the same impact (e.g., Hiebert 1997). Removal of many invasive plants is very expensive and time-consuming (Blossey et al. 2001, Marais et al. 2004), but can have positive outcomes on native species and ecosystems (e.g., Holsman et al. 2010). However, removal of invasive species can have unintended, or even problematic, outcomes (Rinella et al. 2009, Zipkin et al. 2009). Therefore, we need a better understanding of the potential benefits of invasive plant removal on the native flora and fauna.

One very successful invader of the forests and woods of the northeastern and midwestern United States is *Alliaria petiolata* (garlic mustard). *Alliaria petiolata* has spread throughout the northeast and into Canada and the midwestern U.S. from its introduction on Long Island, New York in 1868 (Welk et al. 2002). Little is known about the impacts of *A. petiolata* invasions on the fauna of invaded communities. However, given the ability of garlic